Title Effect of environmental temperature on appetite, energy intake and appetite-regulating hormones during rest

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EFFECT OF ENVIRONMENTAL TEMPERATURE ON APPETITE, ENERGY INTAKE AND APPETITE-REGULATING HORMONES DURING REST

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A thesis submitted to the University of Bedfordshire, in fulfilment of the requirements for the degree of MSc by Research

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Academic Thesis: Declaration of Authorship

I, Rachel Horsfall, declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

Effect of environmental temperature on appetite, energy intake and appetite-regulating hormones during rest

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Rachel Horsfall

Date: 13/04/17

Abstract

There is evidence to suggest that the exercise-induced suppression in appetite is more pronounced when exercise is performed in the heat compared with colder environments. Whether such effects of environmental temperature are seen at rest remains unknown. The aim of this study was to examine the effect of environmental temperature on energy intake (EI), appetite and appetite-regulating hormones during rest. Nine men (aged 21.4 ±1.3 years) rested for 5.5-hours in three conditions i) a thermoneutral environment (20˚C), ii) a hot environment (30˚C) and iii) a cold environment (10˚C). After baseline measures, each participant was supplied with a standardised breakfast meal containing 6 kcal·kg body mass^{-1}. Further blood samples were collected at 0.5, 1, 1.5, 2, 3, 4, 5 and 5.5 h during the postprandial period, with an *ad libitum* pasta meal provided at 4-4.5 h to measure EI. Perceptions of appetite were assessed using 100-mm visual analogue scales every 30 min. Blood samples were analysed for gut hormone concentrations. Significant effects of condition for *ad libitum* EI (P = 0.002) were found; EI was higher in 10˚C and 20˚C compared with 30˚C. The findings of the present study support the limited evidence that environmental temperatures may modulate EI.

Author's declarations

I declare that this thesis is my own unaided work. It is being submitted for the degree of Masters by Research at the University of Bedfordshire. It has not been submitted before for any degree or examination in any other University.

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List of abbreviations

ANOVA – analysis of variance
ARC - arcuate nucleus
CART - cocaine - and amphetamine-regulated transcript
EDTA – ethylenediaminetetraacetic acid
EI – energy intake
GI - gastrointestinal
GLP-1 – glucagon-like peptide-1
GOAT – ghrelin O-acyl transferase
POMC - pro-opiomelanocortin
PFC - prospective food consumption
PYY – peptide tyrosine tyrosine
T_c - core temperature
T_{sk} -skin temperature
VAS - Visual analogue scale

Chapter I

Introduction

Obesity is a major public health challenge both nationally and internationally, with worldwide obesity incidence having doubled since 1980 (WHO, 2015). In 2014, over 1.9 billion adults were overweight, of which 600 million were obese worldwide (NOO, 2016). The most recent statistics from the Health Survey for England show the proportion of the public categorised as obese (BMI 30 kg/m^2 or over) augmented from 13.2% to 24.3% in men and from 16.4% to 26.8% in women from 1993 to 2014 (PHE, 2016, NOO, 2016). Furthermore, the Government's Foresight report has predicted that over half of the United Kingdom (U.K.) adult population could be obese by 2050 (McPherson *et al.*, 2007). Excess adiposity, which characterises obesity, augments the risk of numerous comorbidities, such as cardiovascular disease, musculoskeletal disorders, some cancers, type 2 diabetes and premature death (WHO, 2015). The economic implications of the projected increase in obesity and associated comorbidities are substantial and are set to cost the National Health Service (NHS) £9.7 billion per year by 2050 (WHO, 2015). Such financial costs also encompass indirect costs to the wider economy; for instance, the increase in benefit payments and social care costs. These costs to both society and business are expected to reach £49.9 billion per annum by 2050 (McCormick and Stone, 2007, Butland *et al.*, 2007). Given the rising economic cost of obesity, novel cost effective approaches are needed to treat and prevent excess adiposity.

The fundamental cause of obesity is a continual imbalance of energy intake (EI) exceeding energy expenditure (Goran, 2000, Hill *et al.*, 2012). EI refers to the amount of energy absorbed through food and drink whereas energy expenditure is the amount of energy used i.e. for physical function such as breathing, physical movement and digesting food (Bandini *et al.*, 1990). The consequence of a positive energy balance results in the excess energy stored in the

form of adipose tissue (Jung and Choi, 2014). A successful intervention to regulate body composition must modify the balance between EI and energy expenditure. In this respect, appetite plays an important factor that controls EI and is regulated by numerous physiological, in addition to psychological and social, factors (De Silva and Bloom, 2012).

There is a suggestion that appetite may be affected by environmental temperatures, with exercise performed in hot environments (30-36˚C) promoting marked suppressions in appetite and energy intake when compared to exercise in cold (10-12˚C) and thermoneutral (20-24˚C) environments (Burke, 2001, Wasse *et al.*, 2013, Kojima *et al.*, 2015). However, the effect of environmental temperature with the absence of exercise on appetite has received minimal research attention, meaning that the reported effects may be due to an interaction with exercise and not an independent effect of the environmental temperature. Some evidence has shown that appetite-regulating hormones, such as acylated ghrelin (Tomasik *et al.*, 2005, Wasse *et al.*, 2013) and peptide tyrosine-tyrosine (PYY) (Shorten *et al.*, 2009), are affected by altering environmental temperatures with these hormones having opposing effects on appetite. Acylated ghrelin is known for increasing appetite and subsequently energy intake (Cummings *et al.*, 2001), whereas PYY is associated with decreased energy intake (Batterham *et al.*, 2002, Batterham *et al.*, 2003).

Due to the need for interventions to manage overweight and obesity and the lack of research that has assessed the impact of environmental temperature, the current study aims to investigate the effects of environmental temperature on appetite and appetite-regulating hormones during rest. The results will be used to determine whether manipulations in environmental temperature can be used in overweight and obesity management. Potentially, consuming meals in hotter environments may be beneficial for limiting energy intake and could aid individuals in creating a chronic energy deficit.

Aims

1. To examine the acute effect of environmental temperature on perceived appetite and energy intake.
2. To examine the acute effect of environmental temperature on the acylated ghrelin and total PYY responses.

Chapter II

Literature review

This chapter critically reviews literature investigating the effect of environmental temperatures and exercise on appetite, appetite-regulating hormones and energy intake. The first section provides an overview of energy intake, body weight regulation, appetite regulation, and the interplay with ghrelin (acylated and total) and PYY. Thereafter, the assessment of subjective appetite and energy intake, followed by factors affecting appetite during exercise in different environmental temperatures is discussed. Finally, this chapter examines the effect of resting in varying environmental conditions on appetite, energy intake, and how such alterations may be explained by variance in appetite-regulating hormonal concentrations.

2.1 Energy intake and body weight regulation

A known factor responsible for weight gain and obesity is a positive energy imbalance whereby energy intake continually exceeds energy expenditure (Hill, 2006). It is, therefore, imperative that obesity management considers factors that can influence energy intake, as well as energy expenditure. Studies have found reductions in energy intake through dietary restriction to be effective for attenuating weight gain in order to treat obesity (Ledikwe *et al.*, 2007). As the problem of obesity still exists, research that can help promote reductions in energy intake is required. Importantly, appetite is closely related to energy intake and should be considered when using interventions to control energy intake.

2.2 Regulation of appetite

Appetite is regulated by a multifaceted interaction between metabolic signals produced from the gastrointestinal tract, peripheral organs and adipose tissue, targeting the brain see Figure 1 (Ahima and Antwi, 2008). Appetite can be referred to as feelings such as fullness and the urge to eat food (Blundell, 2006), which can further be manipulated by psychological, physiological

and behavioural influences. The perception of hunger or fullness can be altered by peripheral signals including satiety and adiposity signals which act in the brain; these signals are dependent upon feeding status or responses to experimental manipulations (Blundell, 2006).

Psychological influences on appetite include emotions. For example, emotional stress causes changes in eating with on average a 30% increase of appetite and 48% decrease of energy intake (Macht, 2008). Epidemiological data indicates that stress is associated with an increase in food consumption, with increased levels of stress alluding to an increase in an individuals' drive to eat (Groesz *et al.*, 2012). Stress is also associated with higher energy and fat intake (McCann *et al*., 1990; Kandiah *et al*., 2006), and depression can lead to meal skipping and disorded eating (Fulkerson *et al*., 2004). Further research suggests that mood can alter food choice, and food choice can alter mood (Gibson, 2006). Boredom has also been associated with increase in food cravings and overeating highlighting that boredom is another powerful emotional state motivating food consumption (Hill, Weaver & Blundell 1991; Havermans *et al*., 2015)

2.3 Appetite-regulating hormones

Energy homeostasis is regulated by episodic and tonic hormones providing the brain with acute and chronic nutritional state information, respectively (Williams *et al.*, 2001, Blundell, 2006). Since the nature of appetite is complex, it is important to measure multiple hormones simultaneously to develop a clearer understanding of the direct roles of individual hormones on appetite and energy intake. The gastrointestinal (GI) tract is the largest endocrine organ within the body where acute changes in episodic hormones originate from in response to feeding episodes (Ahlman and Nilsson, 2001, Blundell, 2006). The GI tract is responsible for the secretion of various hormones, both orexigenic (appetite-stimulating) and anorexigenic (appetite–suppressing). Ghrelin is the only known orexigenic hormone (Cummings and

Shannon, 2003), whereas anorexigenic hormones include, PYY, glucagon-like peptide-1, pancreatic polypeptide and oxyntomodulin). While ghrelin increases in anticipation to feeding, the anorexigenic hormones increase in response to feeding (Kreymann *et al.*, 1987, Ahlman and Nilsson, 2001, Batterham *et al.*, 2002, Batterham *et al.*, 2003, Batterham and Bloom, 2003, Martins *et al.*, 2007, Suzuki *et al.*, 2010). These hormones can enter the hypothalamus via an incomplete blood-brain-barrier through the bloodstream, thus allowing interaction with the arcuate nucleus (ARC) (Peruzzo *et al.*, 2000). The ARC is located by the median eminence, regulating a variety of neuroendocrine functions (Bouret *et al.*, 2004) and is an important component of forebrain pathways, playing a critical role in an array of homeostatic circuits. The measurement of blood circulating hormones via neural cells is enabled by the ARC; it also determines the neuronal activity occurring within the hypothalamus (Peruzzo *et al.*, 2000). Within the ARC are neuropeptide Y (NYP) and agouti-related peptide (agRP) neurons; these sensory neurons regulate appetite via increases the desire for food when activated. On the contrary, the activation of pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) are appetite suppressing neurons (Morton and Schwartz, 2001, Morton *et al.*, 2006, Suzuki *et al.*, 2011).

An indirect pathway to regulate appetite is the vagus nerve. The mechanistic action of satiation is affected by vagal sensory information to the brain (Berthoud, 2008). The vagus nerve aids transmissions of gut hormone such as ghrelin, leptin, GLP-1, PYY and pancreatic polypeptide when afferent fibres detect the presence of food within the GI tract (Suzuki et al., 2012, Berthoud, 2008). One example is cholecystokinin (CCK) binding to receptors located on the vagus nerve which consequently activates the hypothalamus via relaying the information (Wren and Bloom, 2007). Through the vagus nerve, CCK and GLP-1 activates the brain stem as the release of nutrients are delivered to the intestines (Naslund & Hellstom, 2007).

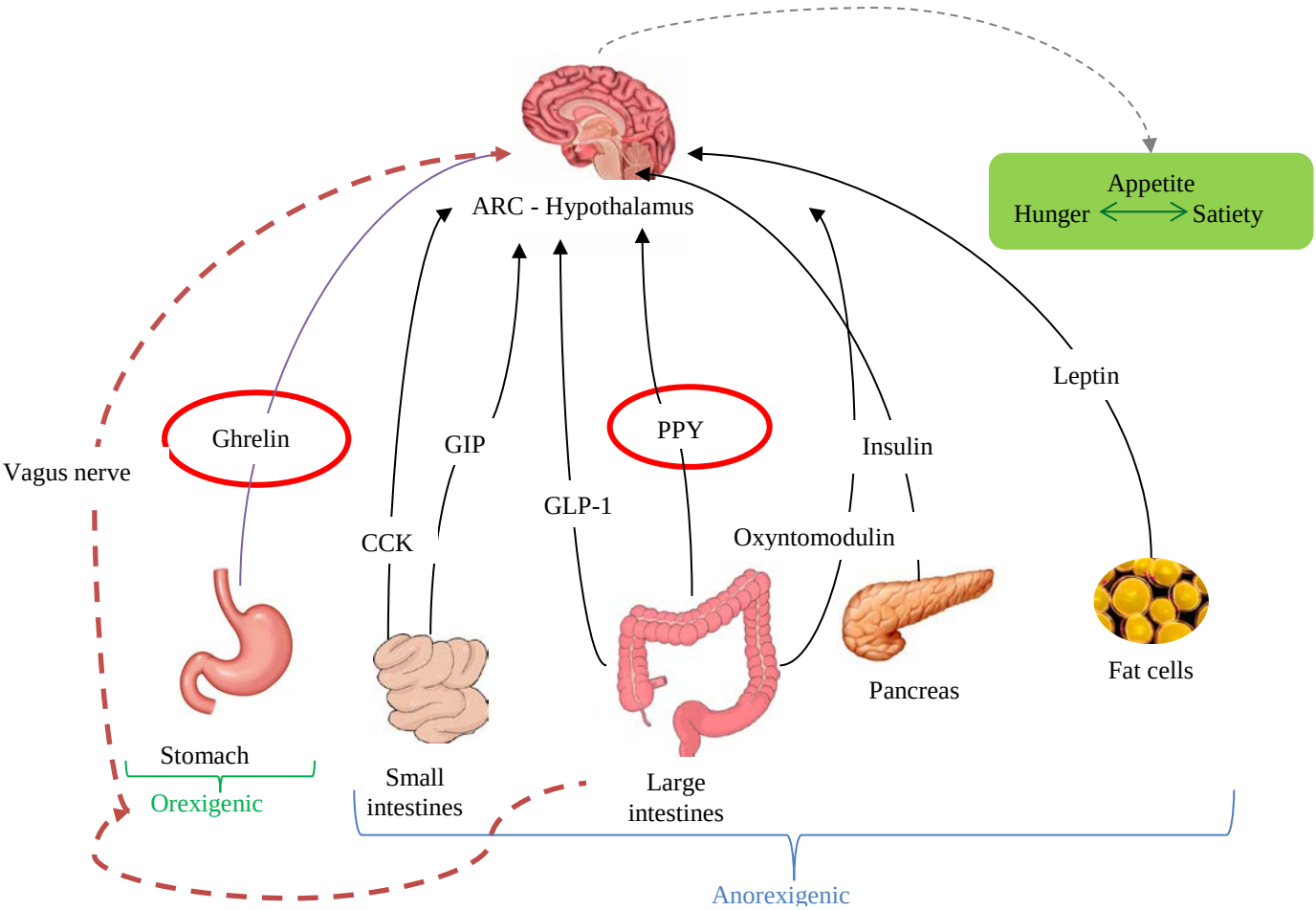

Figure 1 Representation of physiological aspects of appetite and appetite regulating hormones.

Tonic hormones such as leptin and insulin are associated with regulating appetite over a longer period rather than responding on a meal to meal basis (Porte and Woods, 1981, Baskin *et al.*, 1999b, Baskin *et al.*, 1999a). These tonic hormones are positively correlated with adiposity, producing long-term signals in proportion to body fat mass (Garfield *et al.*, 2009). The higher concentrations of leptin and insulin found in obese individuals suggests a resistance to the effects of these hormones to suppress energy intake (Hagobian *et al.*, 2012).

Ghrelin is a peptide hormone synthesised primarily within the stomach, and smaller amounts produced from the intestine, pancreas and other peripheral organs (Gutierrez *et al.*, 2008,

Stengel and Taché, 2012, King *et al.*, 2013b). Ghrelin is encoded by the pre-proghrelin gene and in order to exert its biological functions, post-translational acylation with a medium chain fatty acid is required (Kaiya *et al.*, 2008, Kojima and Kangawa, 2009, Lim *et al.*, 2011); this reaction is catalysed by ghrelin O acyltransferase (GOAT) (Yang *et al.*, 2008, Gutierrez *et al.*, 2008). However, there is little known regarding ghrelin action and the molecular pathways involved. Ghrelin appears in the circulation in two forms: acylated and unacylated. Within the plasma, acylated ghrelin represents ~10% of total ghrelin (Hosoda *et al.*, 2004, Broglio *et al.*, 2004). Acylated ghrelin is able to exert endocrine activities, whereas unacylated ghrelin antagonises the effects of acylated ghrelin on the secretion of insulin and glucose concentrations (Broglio *et al.*, 2004). The main site where ghrelin acts upon is the ARC of the hypothalamus (Olszewski *et al.*, 2003).

Ghrelin concentrations increase preprandially, indicating that it is involved in meal initiation, and decreases to baseline concentrations during the first hour following meal consumption (Cummings *et al.*, 2001). The most important factor for regulating ghrelin secretion has been established as feeding, with total plasma ghrelin concentrations increasing after short-term fasting and decreasing with food intake (Cummings *et al.*, 2001, Williams and Cummings, 2005). The mechanisms mediating these effects are currently unknown, but may be related to nutrient sensing (Tschöp *et al.*, 2000). Despite this, acylated ghrelin is thought to be more pertinent to appetite regulation than unacylated ghrelin as the addition of the medium fatty chain allows it to pass through the blood-brain-barrier and bind to receptors in the ARC, thus increasing appetite (Broglio *et al.*, 2004). Therefore, it is important that acylated ghrelin, rather than total or unacylated ghrelin, is measured within appetite research.

Experiments within mice found that without circadian clock genes, ghrelin is not able to be expressed regularly, suggesting there is a circadian rhythm of ghrelin (LeSauter *et al.*, 2009) whereby concentrations elevate before normal meal times, i.e. early morning, mid-day and evening. Conversely, it has been proposed that food-anticipatory activity is independent of the known circadian clock, although this was reported in mice without a circadian clock ability (Storch and Weitz, 2009). Recently, circadian fluctuations of ghrelin concentrations have been observed in humans, with total plasma ghrelin concentrations increasing prior to meal consumption and during the night (Tsujino and Sakurai, 2012).

2.5 Ghrelin: Energy balance and energy intake

Acylated and unacylated ghrelin concentrations increase with prolonged food deprivation (Zhao *et al.*, 2010). Total ghrelin concentrations are inversely associated with weight gain, adiposity and insulin resistance and positively correlated with weight loss induced by exercise, low- calorie diet and mixed life-style modification (Tschöp *et al.*, 2001b). Indeed, concentrations of acylated ghrelin have been found to be low in obesity (Müller *et al.*, 2010) and even lower in obese binge eaters (Shiiya *et al.*, 2002). Therefore, ghrelin concentrations appear to be affected by energy balance over the long term, with the lowered acylated ghrelin that accompanies weight gain perhaps being an attempt to prevent overeating and restore energy balance (Geliebter *et al.*, 2005). This is further supported by reports that lower ghrelin concentrations were concomitantly found with increased food consumption (Wren *et al.*, 2001). In line with the observation that plasma ghrelin concentrations increase with fasting (Kamiji and Inui, 2008), it has been found that plasma ghrelin concentrations are high in patients with eating disorders, such as anorexia nervosa and bulimia nervosa (Atalayer *et al.*, 2013). It has been established that chronic decreases in energy balance causes ghrelin responses to increase in patients with anorexia nervosa and decrease in obese populations (Tschöp *et al.*, 2001a, Otto *et al.*, 2001, Shiiya *et al.*, 2002). Thus, it can be suggested that ghrelin appears to promote

energy intake and weight gain in conditions of negative energy balance, providing further support that ghrelin is used in the regulation of energy homeostasis in the long term. However, the findings also suggest that ghrelin is not a contributing factor in weight gain as decreased ghrelin concentrations were observed in obese individuals.

The magnitude of the postprandial total ghrelin reduction is proportional to the energy intake and dependent on macronutrient content. Carbohydrates and protein are more effective in suppressing plasma ghrelin concentrations when compared with lipids (Koliaki *et al.*, 2010). When administering total ghrelin, increases in food intake and sensations of hunger occur in various populations including healthy, lean, obese and malnourished individuals (Wren *et al.*, 2001; Müller *et al.*, 2015). Furthermore, intravenous (IV) administration of total ghrelin in healthy participants increases neural activity in specific brain regions in response to pictures of food, suggesting that fasting ghrelin is positively correlated to hunger in response to palatable food stimuli (Kroemer *et al.*, 2013, Holsen *et al.*, 2014, Müller *et al.*, 2015). Findings that ghrelin activates these reward centres suggests that increased food consumption is a more complex mechanism than the physical sensations of hunger or satiety (Malik *et al.*, 2008, Goldstone *et al.*, 2014). Additionally, research has established that increased circulating total ghrelin concentrations results in augmented hunger in preparation for food intake (Perboni and Inui, 2010), whilst concentrations of total ghrelin decrease in response to food intake for 3 hours (Foster-Schubert *et al.*, 2008).

2.6 PYY

PYY is an anorexigenic peptide hormone (Karra and Batterham, 2010). The L-cells located in the distal gut synthesise and release PYY into circulation (Suzuki *et al.*, 2012). Two endogenous forms of PYY exist: PYY_{1-36} and PYY_{3-36}. PYY_{3-36} is the form of PYY involved in appetite regulation and is also the main circulating form (Grandt *et al.*, 1994, Batterham *et*

al., 2006, Korner *et al.*, 2006, Karra and Batterham, 2010, Manning and Batterham, 2014). Similar to ghrelin, PYY targets the neuropeptide Y neurons and exerts its action on the ARC of the hypothalamus (Suzuki et al., 2012). The importance of the Y2 receptor has been investigated by injecting Y2 agonists into the ARC of rats connoting the inhibition of neuropeptide Y via the Y2 receptor is the predominant pathway whereby PYY_{3-36} constitutes as an anorexigenic peptide (Cooper, 2014).

2.6.1 PYY: Energy balance and intake

The inhibition of neuropeptide Y Y2 leads to an increase in satiety signalling. Furthermore, it has been suggested that the regulation of PYY secretion is influenced by feeding (Adrian *et al.*, 1985a). Increased concentrations of circulating total PYY was observed within 15 min of food intake, with 90 min peak concentrations occurring which continued for the remaining 6 hours. The initial increase in total PYY concentrations suggests either a neural or hormonal mechanistic action is involved as nutrients are yet to have reached the L -cells in the GI tract. (Adrian *et al.*, 1985a, Karra and Batterham, 2010). Typically, total PYY concentrations reach a peak 1–2 hours postprandially, with little variation after numerous hours (Adrian *et al.*, 1985b). However, energy intake, the consistency and nutrient content of the food all affect the magnitude and pattern of this postprandial response (Batterham *et al.*, 2006, Helou *et al.*, 2008, Chandarana *et al.*, 2009). For example, it has been previously proposed that dietary fat content stimulates PYY secretion more than protein and carbohydrates (Maljaars *et al.*, 2008). However, it has been found, using perceived satiety, that protein is further satiating than either fat and carbohydrate (Rolls *et al.*, 1988, Weigle *et al.*, 2005).

In humans, post peripheral 90 min administration of PYY_{3-36} decreases energy intake and appetite. These findings have been in both obese and lean individuals (Batterham *et al.*, 2002,

Batterham *et al.*, 2003). A reduction in food intake by 36% occurred after 2h following intravenous PYY_{3-36} administration. The doses were those of similar to post prandial concentrations (Batterham *et al.*, 2002). In a 24h period following the administration total of PYY_{3-36} food intake decreased by 30% with a reduced appetite also established in lean and obese participants. The decrement in food intake observed suggests the obese individuals are not resistant to the effects of PYY and doses within drugs may be an efficient and effective way to counteract obesity (Batterham and Bloom, 2003, Batterham *et al.*, 2003).

Studies have found that obese individuals have lower concentrations of PYY both fasting and postprandial (Batterham *et al.*, 2003, Batterham *et al.*, 2006); obese participants exhibited decreased postprandial PYY concentrations in comparison with lean participants (Batterham *et al.*, 2003). Despite this, obese participants consumed more calories within the test meal (Batterham *et al.*, 2003). However, it is not known whether reduced PYY concentrations are a cause or effect of obesity due to the cross-sectional design of this study.

2.7 The measurement of subjective appetite

Visual analogue scales (VAS) are frequently used to measure subjective appetite, where participants mark within a 100-150 mm line related to descriptive statements, e.g. 'I have never been more hungry'. Multiple scales related to hunger, satisfaction, fullness and prospective food consumption are now employed to take into account the multi-dimensional nature of appetite (Flint *et al.*, 2000, Blundell *et al.*, 2010, Deighton *et al.*, 2013a). It has been suggested that when employing a within subject, repeated measures design, VAS can help to determine where the effect of different treatments can be compared in similar conditions (Stubbs et al., 2000). Importantly, it has been shown that subjective appetite using VAS is correlated with energy intake and is useful when measuring *ad libitum* food consumption and is a valid measurement of appetite (Flint *et al.*, 2000). However, the relationship between appetite is not

always well correlated with energy intake; therefore, it is recommended that both measured food intake and subjective appetite are measured within appetite-related research studies (Stubbs *et al.*, 2000, Gregersen *et al.*, 2008).

2.8 Measurement of food intake

Measuring food intake within a laboratory based setting allows precise measurement of food consumption by recording the weight of food prior and post feeding (Blundell *et al.*, 2009). Conversely, free-living measurements of food intake, by using a food diary for example, allows for the assessment of habitual intakes and thus requires longer duration of recordings (Karvetti and Knuts, 1992). Although external validity is typically greater by assessing free-living diet, this method may lead to participant bias which in turn will decrease the experiments internal validity. Misreporting may occur either to social desirability or to allow for an easier completion for the diary. To ensure a direct and accurate quantification of energy intake, monitoring food intake within a laboratory- based environment is often preferred. Deighton *et al.* (2016) supports this as authors found that pasta *ad libitum* is a valid method of measuring energy intake. Findings suggest that a pleasant-tasting pasta meal incur values of energy intake that are more reflective of appetite ratings (Deighton *et al.*, 2016).

As it has been established the macronutrient composition of food affects appetite, appetite-regulating hormones and energy intake. The consistency of macronutrients should be taken into account when providing *ad libitum* meals to assess energy intake in laboratory settings. For example, acute studies have shown reduced appetite and energy intake with a high protein meal compared with carbohydrate and fat meals (Poppitt *et al.*, 1998, Halton and Hu, 2004), which may be explained by research showing that protein prolongs acylated ghrelin suppression (Bowen *et al.*, 2008).

2.9 Effect of exercise on appetite, appetite-regulating hormones and energy intake

It has been identified that exercise suppresses appetite and does not result in an augmented energy intake acutely. King *et al.* (2010a) used prolonged treadmill running (90 min at 69% of $\dot{V}O_{2max}$) to examine the effects of a large exercise-induced energy deficit of 2935 ± 299 kcal on subsequent appetite and food intake responses for 22.5 h post-exercise. The study found no change in total daily energy intake when compared with a resting control condition. Taking into account the energy expended whilst exercising, an energy deficit still occurred (4912 kJ/1174 kcal) when compared with the control condition (King *et al*. 2010a). Similarly, Douglas *et al.* (2015) found that a single exercise bout induced a relative energy deficit of 4234 kJ (1011 kcal), with no increase in energy intake to compensate for this deficit for up to 48 hours post-exercise. The quantification of food intake during the study utilised two methods: direct laboratory assessment and food diaries (24h after the exercise/control intervention). This was an attempt to ensure that the quantifying process of energy intake was as accurate as possible whilst disrupting the participant's lifestyle to a minimum and allowing a normal daily routine where feasible. This supports previous reports that energy deficits which occur due to exercise do not result in compensatory increases in appetite or energy intake, this being on the day of exercise and up until the morning after. However, a factor that may have affected the variability of the results are the learning effects of the study, where participants may have had knowledge of the authors aims of the study leading to changing normal habits due to social desirability (Douglas *et al.*, 2015). It is possible that energy intake was not quantified accurately due to the use of food diaries, which can be associated with misreporting of dietary intakes, intentional inaccuracies and/or social desirability (Douglas *et al.*, 2015). For future investigations, participants should have little knowledge of the area being investigated where at all possible.

Acute exercise transiently suppresses concentrations of acylated ghrelin. For this response to occur, it has been suggested that the intensity of the exercise bout must be of moderate to high intensity, typically ≥70% $\dot{V}O_{2max}$ (Martins *et al.*, 2008, Stensel, 2010) . The suppression of acylated ghrelin concentrations has been found to last for up to 2 hours post-exercise (Deighton *et al.*, 2013a). This response in acylated ghrelin may partly explain corresponding suppressions in appetite with exercise, a phenomenon known as "exercise induced anorexia", and may also explain why exercise does not result in a compensatory increase in energy intake acutely. Studies employing various designs and measurement periods have confirmed that the energy deficit induced by acute exercise is maintained for up to two days (Douglas *et al.*, 2015). The sustained energy deficit where exercise results in no further energy intake in order to compensate for the energy expenditure may be related to the suppression of acylated ghrelin. Indeed, a single bout of moderate aerobic exercise at 65-75% $\dot{V}O_{2max}$ for a duration of 30-90 min reduces acylated ghrelin concentrations by 14-60% when compared to pre-exercise and a resting condition (Broom *et al.*, 2007, Broom *et al.*, 2009, Balaguera-Cortes *et al.*, 2011, Deighton *et al.*, 2013a, Deighton *et al.*, 2014). Although several studies indicate no effect of exercise upon acylated ghrelin concentrations (King *et al.*, 2010b, Hagobian *et al.*, 2012, Douglas *et al.*, 2015), this may be due to the intensity, mode and duration of the exercise being insufficient to induce an effect.

In literature where both acylated ghrelin and total PYY concentrations have been simultaneously measured it is suggested that endurance exercise (treadmill running for 60 min at 70% $\dot{V}O_{2max}$) results in suppressed acylated ghrelin concentrations for up to 2 h after exercise and elevates total PYY concentrations for up to 3 h post exercise (Broom *et al.*, 2009). Acylated ghrelin has been found to be suppressed following a number of exercise modes e.g. resistance

exercise and aerobic including: cycling, running, and swimming (Broom *et al.*, 2009, King *et al.*, 2010c). It has been implied that running exercise may suppress acylated ghrelin to a greater magnitude than other forms of exercise due to alterations in blood flow to the splanchnic region inhibiting the secretion of ghrelin (Broom *et al.*, 2009, King *et al.*, 2010a). Nonetheless, it has also been suggested that these alterations in blood flow transpire following a cycling exercise bout (Van Wijck *et al.*, 2011, Schubert *et al.*, 2014). Supporting this, no differences in concentrations of acylated ghrelin were found when comparing exercising at the same relative intensity during running and cycling (Schubert *et al.*, 2014,). More specifically, 90 min of resistance exercise has been found to suppress acylated ghrelin concentrations similarly to 60 min of a treadmill exercise bout (Broom *et al.*, 2009). This study also demonstrated hunger suppressions with resistance exercise, again suggesting acylated ghrelin may possibly promote exercise-induced anorexia. Furthermore, total PYY concentrations were elevated until meal consumption at 2 h following resistance exercise (Broom *et al.*, 2009). Swimming (60 min) has also been shown to suppress acylated ghrelin and appetite concomitantly; however, no significant correlations were found, suggesting there may not be a strong association between acylated ghrelin and subjective appetite (King *et al.*, 2010c). However, PYY was not measured in this study; therefore, it is possible that this appetite-regulating hormone may be related to subjective appetite. Further findings indicate that swimming induces a lesser suppression of PYY concentrations compared to running due to the lack of increase in body temperature being suggested as a contributing factor (Russell *et al.*, 2009). This leads to suggest that body temperature was not altered enough to have an effect on acylated ghrelin concentrations and that environmental temperature may affect appetite regulation.

Like ghrelin, exercise induced changes in PYY may also partly explain the suppression in appetite with acute exercise. Both moderate (50% $\dot{V}O_{2max}$) and high intensity (75% $\dot{V}O_{2max}$)

exercise for 30 min has been shown to induce acute increases in PYY_{3-36} (Ueda *et al.*, 2009). Even so, research has been equivocal, with some studies reporting an increase in PYY following exercise at (65% $\dot{V}O_{2max}$) (Deighton *et al.*, 2013b) while others report no change following exercise at 70% HR_{max} (Martins *et al.*, 2014). Such disparate findings may be partly due to the possibility that high-intensity exercise may influence total PYY rather than PYY_{3-36} concentrations, as high-intensity exercise of 30 min demonstrated increases in total PYY (Beaulieu *et al.*, 2014) whilst others show no change in PYY_{3-36} concentrations (Deighton *et al.*, 2013a, Sim *et al.*, 2014, Metcalfe *et al.*, 2015).

Overall, the literature proposes that acute exercise, particularly high intensity exercise, suppresses acylated ghrelin concentrations whilst increasing PYY concentrations (Martins *et al.*, 2007, Ueda *et al.*, 2009, Broom *et al.*, 2009, Deighton *et al.*, 2013a, Hazell *et al.*, 2015); this can result in exercise induced energy deficits with individuals failing to compensate through increases in energy intake (King *et al.*, 2010a, Douglas *et al.*, 2015). However, exercise-induced anorexia and appetite-regulating responses may be influenced by factors other than the exercise characteristics. There is some research to date that has shown the extent of the suppression in appetite seen with exercise is dependent on the temperature of the environment where the exercise is conducted.

2.10 Exercise and appetite in varying environmental temperatures

Some literature suggests that the effect of exercise on appetite and responses of appetite-regulating hormone are affected by environmental temperature. The application of such findings appears to be promising, with temperature being a particularly feasible and practical environmental factor to manipulate when compared with altitude, for example (White *et al*,

2005, Crabtree and Blannin, 2015, Wasse *et al.*, 2013). Despite this, empirical evidence on the effect of hot and cold temperatures on appetite and the exact mechanisms involved is sparse.

Appetite has been reported to be suppressed in the heat (30˚C) during exercise (Burke, 2001). However, it is evident that appetite responses in the heat are yet to be well established and are mainly based on anecdotal evidence. A mediating role of appetite-regulating hormones in responses of appetite to changes in environmental temperatures has been suggested, but there is little research to supports this (Shorten *et al.*, 2009, Wasse *et al.*, 2013). Furthermore, alternative physiological changes may be involved.

A thermoregulatory response during exercise in the heat induces blood flow to the skin in order to maintain body temperature (Robinson *et al.*, 1965). Homeostasis also induces blood flow redistribution to the working muscles and the brain in order to maintain energy metabolism and the functioning of the CNS (Cheuvront *et al.*, 2010). The thermal and metabolic demands must be met by the circulation of the blood and are influenced by the intensity and duration of exercise; an increased exercise intensity whilst in the heat leads to a reduced blood flow to the sphlanchnic tissues (Rowell *et al.*, 1966). It has been proposed that blood flow redistribution away from the gut that occurs during exercise in a hot environment may mediate the reduction in appetite and energy intake (Rowell *et al.*, 1965, Rowell *et al.*, 1966, Rowell, 1974, Ho *et al.*, 1997). Research has suggested that the reductions of blood flow to the splanchnic region may in turn affect the suppression of acylated ghrelin whilst exercising (Broom *et al.*, 2007), with some authors suggesting that the reduction in oxygen delivery interferes with ghrelin secretion, leading to a decreased proportion of total to acylated ghrelin (Broom *et al.*, 2007). Therefore, it is possible that appetite and appetite-regulating hormone concentrations are attenuated to a greater degree during exercise in the heat, or even whilst resting in the heat when compared with colder environments. However, no study has investigated whether changes in appetite-

regulating hormone concentrations occur whilst resting in the heat, and only a small number of the studies involving exercise in different environmental temperatures have been conducted.

Shorten *et al.* (2009) examined the effects of exercising in a hot environment and its effect on appetite-regulating hormones. The study compared the regulation of appetite during three conditions: 40 min of exercise at 70% $\dot{V}O_{2max}$ in 36˚C and 25˚C, and rest in 25˚C. The findings showed that in comparison with a resting control (25˚C), exercise in 36°C caused significant reductions in relative energy intake (REI), defined as the energy cost whilst exercising in relation to energy intake. Furthermore, REI was greater post-exercise at 25°C compared with control, but was similar between exercise at 36°C and the exercise at 25˚C condition. An increase in total PYY concentrations was observed during exercise in the heat compared with the resting control condition prior to the *ad libitum* meal, this may have mediated the findings of reduced REI. (Shorten *et al.*, 2009). Whilst there was no significant difference in acylated ghrelin concentrations immediately preceding the meal between either of the exercise conditions, there was a tendency for a reduced acylated ghrelin concentration with exercise in 36˚C compared with both the resting and exercise 25˚C conditions. This suggests that environmental temperature may affect appetite through changes in both PYY and acylated ghrelin concentrations despite the participants only being exposed to each temperature for only 40 min (Shorten *et al.*, 2009). The authors postulated that the observed decrease in REI after exercising in a hot environment may have been attributed to the hot temperature itself as the exercise 25˚C condition did not affect REI. However, a direct comparison of resting in the heat compared with 25˚C would be needed to confirm the independent effect of environmental temperature on appetite and energy intake. Additionally, the study had various limitations. Subjective appetite was not recorded and the measurement of energy intake was recorded once, i.e., immediately after exercise. Indeed, it is possible that energy intake in subsequent meals

may have shown compensatory effects and not resulted in significant exercise-induced energy deficits. Nevertheless, these limitations were taken into account in some of the subsequent studies to be discussed (e.g., Wasse *et al.* (2013)).

Similar studies investigating the effects of environmental temperature on appetite have used submerged exercise. Exercise (submersed cycling at 60% $\dot{V}O_{2max}$ for 45 min) in cold water (20˚C) found an elevation in energy intake 1 h post- exercise by ~45% when in comparison with exercising in neutral water (33°C) (White *et al.*, 2005). Energy intake was measured via unlimited access to an assorted buffet for 60 min. Although this was within an isolated food consumption area, free from social cues, the participants reported an awareness of their energy intake being measured, meaning that the responses may have been influenced by social desirability. The results indicated that exercise in cold water stimulated energy intake, a finding supported by Dressendorfer (1993) where participants consumed a significantly increased energy intake, via a buffet meal, in a cold water cycling condition (30 min modified cycling in 22°C cold water) compared to warm water cycling (34°C), cycling on land and a resting control condition.

The mechanistic action governing the responses of appetite regulating hormones remain unclear. However, White *et al.* (2005) suggested that the blood flow redistribution could be partly responsible. When comparing exercise in the water with air, exercise in the cold water may not reduce blood flow to the splanchnic region to the same magnitude as exercise in cold air. This may be due to a greater magnitude of cold induced cutaneous vasoconstriction occurring as the conduction of the water would be greater than when in air, possibly leading to a lower skin temperature. This may lead to a larger splanchnic blood flow as there has been a larger quantity of blood shunted from the skin in the cold water compared to the cold air. Therefore, during exercise in cold air compared with cold water, an increase in blood

availability to circulate appetite-regulating hormones, such as acylated ghrelin and PYY may occur. However, the authors did not measure concentrations of appetite-regulating hormones in this study.

It has previously been demonstrated that concentrations of acylated ghrelin are augmented immediately after neutral water immersion compared with a colder control, as no concentration differences of acylated ghrelin were found in response to cold water immersion (Halse *et al.*, 2011). The implications of these findings suggest that thermoneutral environments increase concentrations of ghrelin compared to cold. The disadvantages of investigating environmental temperature in water compared with air include the duration of the exposure, which is often limited, and the timing involved with keeping the same temperature throughout to ensure consistency. Additionally, the scope to take blood samples at multiple time points is limited. Overall, the research advocates that the response of appetite-regulating hormones to different environmental temperatures remains elusive.

A study in overweight men and women examined the effects of exercising in varying ambient temperatures on appetite and energy intake (Crabtree and Blannin, 2015). After a 45 minute baseline period at room temperature, participants were exposed to the environmental temperature (cold and thermoneutral) throughout a walking bout (45 min brisk walk at 60% $\dot{V}O_{2max}$). They then returned to room temperature for a further 45 min and were subsequently exposed to a 30 min *ad libitum* buffet. Results indicated that exercise in a cold (8˚C) environment resulted in increased post-exercise energy intake when compared with exercise in a thermoneutral environment (20˚C) (Crabtree and Blannin, 2015). Furthermore, the authors established that in comparison to the neutral condition concentrations of acylated ghrelin increased immediately after the exercise bout in the cold and remained elevated until the cessation of the condition (40 min post exercise). Additionally, no differences were observed in PYY concentrations after exercise until the end of the condition in the cold compared to the

thermoneutral environment. Thus, increases in energy intake post exercising in a cold environment may be mediated by increased concentrations of acylated ghrelin and deductions in total PYY concentrations.

However, as the study did not compare with either a resting control or hot condition it cannot be concluded if the reported effects were due to the combined effect of exercise and the cold or an independent effect of the colder environment, or whether hot exposure could aid weight management by reducing energy intake. Furthermore, as subjective appetite was not measured and energy intake was only measured once (buffet meal 45 min post- exercise), the authors suggested that future studies using visual analogue scales are required to determine subjective appetite changes throughout exposures to different environmental temperatures in more detail. A similar study in overweight/obese participants compared the influence of exercise (30 min cycling at 65% of $\dot{V}O_{2max}$) performed in three different environmental temperatures ranging from 36˚C (hot), to 24˚C (neutral), and 12˚C (cool) (Kojima *et al.*, 2015). In contrast to previous studies (Wasse *et al.*, 2013, Crabtree and Blannin, 2015), the results indicated no effect of temperature on exercise-induced plasma acylated ghrelin and PYY responses. However, unlike the other studies, the authors did not measure energy intake and the study trial conducted only lasted 30 min. A longer exposure may have increased the likelihood of temperature exerting effects on the outcomes. The authors did, however, report that the suppression in subjective feelings of hunger, induced by exercise, was decreased during exercise within the cool condition compared with hot and neutral, supporting the findings of previous research (Crabtree and Blannin, 2015, Wasse *et al.*, 2013). A limitation of this study was the absence of energy intake data, thus it is not possible to determine whether suppressions in subjective hunger resulted in reductions in energy intake.

Using a longer condition duration (7 h) than the previous studies discussed, Wasse *et al.* (2013) compared the effects of exercising in different environmental temperatures on the regulation of appetite using two separate studies, with similar samples in each (recreationally active, healthy males). Both studies used a 60 minute treadmill run at a speed that elicited 65% $\dot{V}O_{2max}$ followed by 6 h rest: the first study (Study 1) compared the effects of exercise at 30°C versus 20°C, and the second study (Study 2) compared exercise at 20°C versus 10°C. Participants were exposed to the environment for the full 7 h. The concentrations of acylated ghrelin did not differ throughout the conditions in either study 1 or study 2. However energy intake, which was quantified via two *ad libitum* meals at 2 h and 5.5 h, decreased post exercise in the heat (30˚C) during study 1 and increased post exercise in the cold condition (10˚C, Study 2). However, it was not possible to directly compare the hot and cold conditions due to the inclusion of different participants for the two studies. Thus, any differences between the conditions may have been influenced by between-group differences in exercise responses, appetite regulation and eating behaviour. For example, regarding the *ad libitum* meal, eating at a moderate pace has been found to lead to a more pronounced anorexigenic gut peptide response than eating fast (Kokkinos *et al.*, 2010).

A further limitation of the study was the lack of the measurement of the postprandial response of the anorexigenic appetite-regulating hormones, for example PYY, which could provide a further mechanistic understanding of differences in energy intake between the conditions. Furthermore, as participants rested in the environment that the exercise was completed in (i.e. hot, neutral or cold) for 6 h post-exercise, it was not possible to isolate the effects of environmental temperature from those of exercise on appetite regulation. Indeed, it is plausible that differences in appetite and energy intake between the conditions could be due to the participants being exposed to that environment during the post-exercise rest period, or could

be due to an interaction with exercise. Different responses may occur at rest due to the blood flow redistribution, which may not be as pronounced during rest in the heat compared with exercise in the heat. Further research where environmental temperature is manipulated during rest would be useful in determining the independent effect of environmental temperature on appetite, regardless of any interactions with exercise.

2.11 Effect of environment on appetite during rest

Epidemiological data has examined ambient temperature and its association with the prevalence of obesity within the Korean population, showing that BMI and waist circumference are invertly correlated with mean annual temperature (6.6˚C to 16.6˚C) (Yang *et al.,* 2015). Similar findings have been observed in England, comparing residing temperatures of above 23˚C to below 19˚C implying that higher indoor temperatures could reduce BMI levels (Daly, 2014). These findings suggest that the environmental temperature has an association with BMI, with further research needed to establish the causal nature of this relationship.

Acute experimental studies have reported that ambient temperatures affect energy metabolism. However, very few studies have investigated the effects of different environmental temperatures on energy expenditure, and the subsequent impact on energy intake. One study examined the effects of a lowered ambient temperature of 16˚C compared with 22˚C (Westerterp-Plantenga *et al.*, 2002). Participants stayed within a respiration chamber for 60 h for each condition executing standardised daily activities and energy intake was determined by energy requirement in the first 24 h and *ad libitum* energy intake during the second 24 h. The authors reported that energy intake increased at 16˚C (134 ± 14%) compared to 22˚C (132 ± 12%) alongside a significantly increased subjective appetite, concluding that overeating at a lower temperature compensates for an increased energy expenditure as well as attenuating the decrease in core body temperature (Westerterp-Plantenga *et al.,* 2002).

More recently, a study examined the effects of a single 2.5 h episode of either mild cold (18˚C) or thermoneutralality (24˚C) on hunger, food intake and satiety in men and women (Langeveld *et al.*, 2016). Metabolic rate, skin temperature, cold and hunger scores were measured every 30 min followed by an *ad libitum* meal post- cold exposure. During mild cold exposure feelings of hunger increased compared to the thermoneutral condition; however, once out of the cold, differences were not found. Thus, in contrast to the 48 h exposure conducted by Westerterp-Plantenga *et al.* (2002), short-term mild cold exposure resulted in an increase in energy expenditure that was not directly compensated by an increase in energy intake.

Exposure to hot and cold temperatures has been found to influence gut hormone responses. Tomasik et al (2005) used 17 healthy males to rest for 30 minutes at 2°C, 20°C and 30°C. Plasma total ghrelin was measured and the results demonstrated that total ghrelin elevated in 2°C compared with 20°C and decreased after a 30°C exposure compared to 20°C. The results indicate that within a cold environment the increase in appetite that occurred may be mediated by the secretion of ghrelin. Alongside the significantly suppressed total ghrelin concentrations within the hot environmental compared to neutral it can be suggesting that the temperature of the surrounding environment may be mediated by the changes in ghrelin concentration. Nevertheless, the study did not measure subjective appetite, therefore it is speculation that the changes in total ghrelin concentrations would have resulted in changes within subjective appetite in the different environments. Furthermore, the study measured total ghrelin not acylated ghrelin and as AG only represents ~10% of total ghrelin and increase in endocrine activities may not have occurred.

2.12 Summary

Appetite is becoming an increasingly pertinent topic to investigate due to the increasing health concerns of overweight and obesity. Evidence suggests that the environmental temperature can affect appetite. However, a limited amount of research to date has investigated the impact of environmental temperature on appetite responses during both rest or with the addition of exercise. The observations suggest that cold environments result in orexigenic responses compared with thermoneutral and hot environments. However, the independent effect of the environmental temperature with the absence of exercise on appetite regulation and energy intake requires elucidating. It is important to determine whether environmental change in the absence of exercise may still suppress appetite or whether the appetite responses are exclusively an exercise-specific response. Therefore, the aim of this study is to determine the effect of environmental temperature on subjective appetite, appetite-regulating hormones, and energy intake during rest to help establish the independent effect of environmental temperature on appetite regulation.

Table 1 Literature review table

Study	Environmental Conditions	Exercise	Effects on appetite & EI	Effects on total PYY	Effects on ghrelin (AG &Total)
White *et al*. (2005)	20°C (cold water) vs. 33°C (neutral water)	45 min submersed cycle (60% $\dot{V}O_{2max}$)	↑ EI by ~45% vs. neutral	N/A	N/A
Shorten *et al. (*2009)	36˚C, 25˚C and rest at 25˚C	40 min run (70% $\dot{V}O_{2ma}$)	↓REI in 36˚C vs. rest. ↑REI at 25˚C vs. rest; ↔ 36˚C vs. 25˚C	↑Total PYY in 36˚C vs. rest	↔ AG between 25˚C and 36˚C
Wasse *et al*. (2013)	20˚C vs. 30˚C (Study 1); 20˚C vs. 10˚C (Study 2)	60 min run (65% $\dot{V}O_{2ma}$) 6h rest, *Ad libitum* at 2 h and 5.5 h	↓ EI & hunger in 30˚C vs. 20˚C; ↑ EI post exercise; ↓ satisfaction and fullness in 10˚C vs. 20˚C	N/A	↔ AG
Kojima *et al*. (2015)	36˚C vs. 24˚C vs. 12˚C	30 min cycling (65% set to 70rpm)	↓ hunger in 36˚C and 24˚C vs. 12˚C	N/A	N/A
Crabtree and Blannin (2015)	8˚C vs. 20˚C	45 min walking at 60% $\dot{V}O_{2ma}$	↑ EI in 8˚C vs. 20˚C	↔ PYY post exercise in 8˚C vs. 20˚C	↑ AG post exercise until cessation in 8˚C vs. 20˚C
Tomasik *et al*. (2005)	2˚C, 20˚C and 30˚C	N/A 30 mins rest	↑ hunger in 2˚C	N/A	↑ Total ghrelin in 2˚C vs. 20˚C ↓ total ghrelin in 30˚C vs. 20˚C
Westerterp-Plantenga *et al*. (2002)	16˚C vs. 22˚C respiration chamber for 60 h		↑ EI 16˚C (134 ± 14%) vs. 22˚C (132 ± 12%); ↑ subjective appetite	N/A	N/A

Chapter III

Methodology

This study was approved by the Institute of Sport and Physical Activity Research Ethics Committee at the University of Bedfordshire.

3.1 Participants

In order to determine the number of participants required for this study, power calculations using G*Power were carried out based on previous data by Wasse *et al,* (2013), who reported that subjective appetite (prospective food consumption (PFC)) was significantly elevated at baseline in the cold when compared with temperate conditions (cold 73 ± 3 mm, temperate 66 ± 3 mm; $P < 0.05$). Based on this, the number of participants required to detect a minimum expected difference in appetite between different environmental conditions in this study at 85% power and significance level of 0.05 was ten. To account for drop-outs, twelve males (aged 20 to 24 years) were recruited for the study. However, three participants withdrew from the study during the course of data collection. Participants withdrew due to the following reasons: Timings did not fit in well with dissertation and exam time; fainted several times during blood draws and collapsing veins due to taking part in other studies involving blood collection. Complete datasets were available for a total of nine participants.

In order to increase the likelihood of finding a true association between exposure/intervention and outcomes and to reduce risks to participants, the following inclusion criteria needed to be met: male, recreationally active, non-smoker, not obese (body fat percentage <20%; body mass index (BMI) <30 $kg \cdot m^{-2}$), no known cardiovascular disease or abnormalities, not taking medication that influenced metabolism, habitual breakfast eater. The use of males in this study was due to the menstrual cycle of women and how that has an effect on their appetite

throughout the cycle. Research has shown that there is a plausible link between the menstrual cycle and food consumption as macronutrient intake was higher during the luteal phase of the menstrual cycle over the course of two menstrual cycles in 259 healthy women (Gorrczyca *et al.,* 2016). The differences in physiology of eating and in circulatory levels of hormones in men and women with further changes depending on the state of menstruation was the reasoning for women being omitted from the study. The participant characteristics are described in Table 2.

Table 2 Physical characteristics of study participants

Characteristic	Mean ± SD
Age (y)	21.4 ± 1.3
Height (m)	178.6 ± 5.1
Body mass (kg)	74.9 ± 18.1
Body fat (%)	15 ± 3.2

Values are mean ± SD (n = 9)

3.2 Procedure

Prior to data collection, all participants were given an information sheet (Appendix A) outlining the requirements of the study. Any further information required for the participants was provided verbally before they signed an informed consent sheet (Appendix B) agreeing to partake in the study. A Pre-test Medical Questionnaire (Appendix C) and a blood screening form (Appendix D) were completed prior to any data collection to screen participants for any potential factors that may affect their eligibility to participate, e.g. blood borne diseases. A breakfast habits questionnaire (Appendix E) was also completed to determine participants were all habitual breakfast eaters. The participants were made aware that they were able to withdraw from the study at any point.

3.2 Experimental Design

Using a repeated measures cross-over design, participants completed three 5.5-hour experimental trials in an environmental chamber: thermoneutral environment (20˚C) (CON), hot environment (30˚C) (HOT), and cold environment (10˚C) (COLD). The three conditions were completed in an incomplete counterbalanced order. The temperatures were similar to those used in previous research that found differences in appetite according to environmental temperature (e.g., Kojima et al. 2015; Wasse et al. 2013). There was a 7-14 day wash out period between the experimental trials to reduce any potential carryover effects. Trials took place at the same time of day to minimise the influence of circadian variation and to mimic a routine that would reflect expected habitual breakfast (~09:00) and lunch (~13:00) timings.

3.2.1 Calculations

Mean skin temperature was calculated using the following equation:

$$T_{SK} = 0.3*(T_{arm}+T_{chest}) + 0.2*(T_{calf}+T_{thigh})$$

Body heat content was calculated using the following equation:

$$Q_{BHC} = m \times c \times T_b$$

Where m = body mass, c = specific heat of the body (3.48 kJ. $kg^{-1.}{}^{\circ}C^{-1}$) and T_b = mean body temperature.

$$T_b = 0.79(T_{re}) + 0.21\ (T_{sk})$$

3.3 Preliminary measures

Prior to experimental trials, anthropometric measures of height (Stadiometer, Harpenden, HAR- 92.602, Holtain, Crymych, Wales), body mass (Tanita, BWB0800, Amsterdam, The Netherlands) and percentage body fat (via air displacement plethymography) (Bod Pod, 20000A, Cosmed, Middlesex, UK) were recorded. The participants were also familiarised with study procedures and equipment.

3.4 Pre- trial lifestyle controls

Prior to each main trial, participants were instructed to refrain from alcohol and caffeine consumption and to have not taken part in any strenuous physical activity for a period of 24 h. Participants were also asked to complete a 24 h weighed food diary prior to their first main trial and not to eat after 21:00; participants replicated their dietary intake (quantity and timings) in the 24 h period before subsequent main trials (Appendix F). Each participant consumed 500 ml of water (this equated to ~ 5-7 mL/kg body mass) 2 h before arriving to the laboratory to promote a euhydrated state (Sawka *et al.*, 2007).

Participants were advised before each trial what condition they would be completing so that clothes could be chosen accordingly. Thus, clothing was not standardised between the experimental conditions. Participants were informed of the order of their conditions and, thus the environmental temperature, during preliminary testing.

3.5 Experimental Protocol

The experimental protocol is shown in Figure 2. On the morning of main trials, participants arrived at the laboratory at 08:30 in the fasted state. On arrival, participants were fitted with a heart rate monitor (Polar, FS1, Kempele, Finland), skin temperature (T_{sk}) thermistors (Grant, EUS-UVS5-0, Wessex Power, Dorset) located on the upper arm (T1), chest (T2), thigh (T3) and calf (T4) using adhesive tape, and a rectal thermometer (YSI, 401, Yellow springs, Ohio)

inserted 10 cm past the anal sphincter to monitor core temperature (T_c). A urine sample was collected to confirm that the participant was euhydrated (Atago Vitech scientific, Pocket PAL-OSMO, HaB Direct, Warwickshire), i.e., urine osmolality < 700 mOsm/kg (Sawka *et al.*, 2007). Subsequently, an IV catheter was inserted into an antecubital vein by a trained researcher (with an up-to-date hepatitis B vaccination) and two fasting baseline blood samples were collected 5 min after the insertion of the cannula within a thermoneutral environment. Participants then entered the environmental chamber, which was set at 10˚C, 20˚C or 30˚C depending on the condition. After 5 min, each participant was supplied with a standardised breakfast meal containing 6 kcal·kg body mass^{-1}. Blood samples were then collected at 0.5, 1, 1.5, 2, 3, 4, 5 and 5.5 h during the postprandial period. Participants were free to complete work on laptops whilst remaining seated at all times. An *ad libitum* pasta meal was provided at 4-4.5 h (all food was consumed in the simulated environmental conditions of each main trial). Perceptions of hunger, satisfaction, fullness and prospective food consumption were assessed using 100-mm visual analogue scales (VAS; Appendix G) in the fasted state and postprandially every 30 min. Overall appetite score was calculated as the mean value of the four appetite perceptions after inverting the values for satisfaction and fullness (Stubbs et al., 2000b). Humidity was controlled at 50% RH for all conditions in line with previous studies (King *et al.*, 2010a, Wasse *et al.*, 2013). The temperature and humidity of the environmental chamber were recorded at the same time as each VAS. Throughout the trials HR, Tc, Tsk was measured every 10 min to one decimal place.

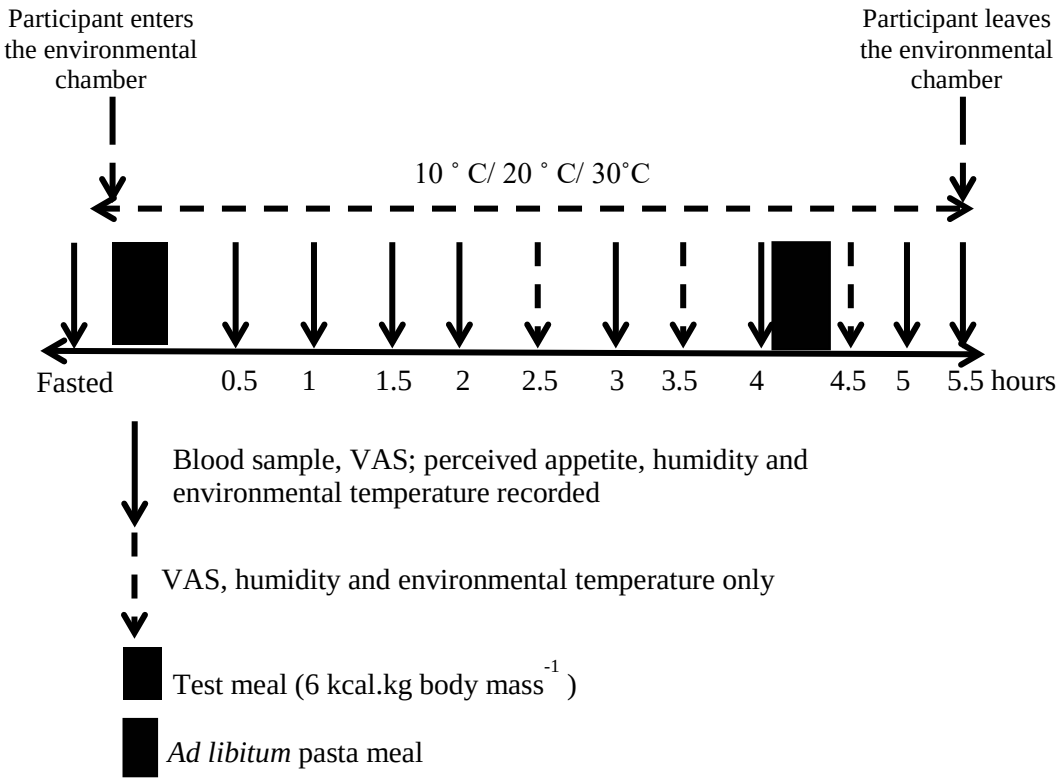

Figure 2 Experimental protocol

3.6 Fluid Consumption

During the trial participants were allowed to consume water *ad libitum* for each condition. Water consumed throughout each condition was recorded. Body mass was measured in a fasted state at the start of the trial and at the end of the trial cessation to ensure hydration by monitoring potential fluid loss.

3.7 Blood Sampling and Chemistry

Blood samples were collected into pre- chilled EDTA vacuettes (VACUETTE, Greiner Bio-One, Austria). From each sample, 50-µL blood samples were collected into two heparinised microhaematocrit tubes for determination of haematocrit and a 20-µL sample into a microcuvette for determination of haemoglobin concentrations to assess changes in plasma volume (Dill and Costill, 1974). One vacuette was immediately centrifued at 1500 × *g* for 10 min at 4°C (Heraeus Multifuge X3R, Thermo Scientific, Loughborough,UK). The plasma

supernatant was placed into separate cryovials and stored at −80°C until later analysis of total PYY. To prevent the degradation of acylated ghrelin, a solution of potassium phosphate buffer (PBS), P-hydroxymercuribenzoic acid (PHMB) and sodium hydroxide (NaOH) (this was 10μL per mL of blood) was added to one EDTA vacuette. This vacuette was then spun in a refrigerated centrifuge at 1500 × g for 10 min at 4 °C. The plasma supernatant was then placed into a storage tube and 100 μL of hydrochloric acid (HCl) per 1 mL of plasma was added to preserve acylated ghrelin (Hosoda *et al.*, 2004).Thereafter, the sample was spun at 1500 × g for 5 min at 4 °C prior to storage at −80 °C until later analysis of acylated ghrelin.

Commercially available enzyme immunoassays were used according to manufacturer's instructions to determine plasma concentrations of acylated ghrelin (SPI BIO, Montigny le Bretonneux, France) and total PYY (Millipore,Watford, UK). To eliminate interassay variation, samples from each participant were analysed in the same run. The Intra assay coefficient of variation was 3%.

3.8 Test meals

Participants were provided with a standardised breakfast meal consisting of bread, orange juice, milk, cheese, and jam: 17% protein, 33% fat, and 46% carbohydrate. This was similar to a previous study (Martins *et al.*, 2012). The meal contained 6 $kcal.kg^{-1}$ of body mass (Table 3). Participants were instructed to consume the meal within 10 min. The consumption time of the breakfast was recorded and participants were instructed to replicate this in subsequent trials. The *ad libitum* pasta meal provided at 4-4.5 h consisted of penne pasta (uncooked 500 g) (Tesco, Dundee) and chunky vegetable tomato sauce (500 g) (Tesco, Dundee) (total energy content: 8,326 kJ (1990 kcal)) cooked and prepared according to manufacturer's preparation instructions. In all trials, the participants had 30 min to consume the *ad libitum* pasta meal. The *ad libitum* meal was presented in the same way for each trial and each participant. The pasta

was presented in a standardised large bowl and with a standardised spoon. A standardised plate and cutlery (fork, spoon and knife) was also provided and participants were instructed to serve themselves the amount desired onto the plate and to eat until they felt satisfied. Participants were not observed during when consuming the *ad libitum* meal. The pasta was weighed before and after using digital scales to determine food consumption in grams (Salter, HoMedics Group Ltd, UK); energy intake was calculated from the grams of food consumed.

Table 4 Example breakfast (for a 75 kg participant)

Breakfast	**Quantity (g)**	**Energy (Kcal)**	**CHO (g)**	**Fat (g)**	**Protein (g)**	**CHO (%)**	**Fat (%)**	**Protein (%)**
Kingsmill 50/50[a]	61.4	142.5	23.7	1.1	5	21	2.2	4.5
Pure orange juice [b]	75	35.3	7.9	0.1	0.4	7	0.1	0.3
British semiskimmed milk [b]	80.4	40.2	3.9	1.4	2.9	3.4	2.9	2.6
British medium[b] cheddar [b],	40.2	167.1	0	14.0	10.2	0.04	28	9.1
Strawberry jam [b]	25	65	15.9	0.03	0.1	14.1	0.05	0.1
Total	**282**	**450**	**51**	**17**	**19**	**46**	**33**	**17**

Abbreviations: CHO, carbohydrate

[a] Kingsmill, Maidenhead, UK.

[b] Tesco, Dundee

3.9 Statistical Analyses

SPSS version 22 (SPSS inc., Chicago, IL.) was used to complete all statistical analyses. Descriptive data are represented as mean ± standard deviation (SD). Normality of distribution was checked using Q-Q plots and deemed plausible for each variable prior to analyses. Two-way analysis of variance (ANOVA) with repeated measures were used to determine differences in appetite-regulating hormone concentrations, subjective appetite, HR, T_{re} and T_{sk}, body heat

content between the three conditions and over time. Appetite hormone data is presented as delta values. One-way ANOVA were used to assess *ad libitum* and urine osmolality between conditions. Body weight changes were analysed using a one-way ANOVA. Bonferonni post-hoc corrections were used to examine differences between individual conditions and time points for significant main effects. Where significant interactions were found, between-condition differences at each time point were examined using one-way ANOVA with Bonferroni post hoc correction. Assumptions of homogeneity of variance were assessed using Mauchly's test of Sphericity and a Greenhouse – Geisser correction was applied to the degrees of freedom if the spherecity assumption was violated. Statistical significance was accepted as $p \leq 0.05$.

Chapter IV

Results

A table of descriptive statistics for all outcome variables is now included in the appendix of the thesis.

4.1 Subjective appetite

Hunger: There was no main effect of condition on hunger ($F_{2,16} = 3.58$, $p = 0.90$). There was a main effect of time on hunger ($F_{8,64} = 5.62$, $p < 0.01$) and an interaction effect between time and condition was found ($F_{16,128} = 3.80$, $p < 0.01$). Post-hoc analysis of between-condition differences at each time point were examined using a one-way ANOVA with Bonferroni post-hoc correction and found no differences between the three conditions at each individual time point ($p \geq 0.10$); see Figure 2.

Prospective food consumption: No significant main effect of condition ($F_{2, 16} = 7.24$, $p = 0.51$) was found. A main effect of time was found ($F_{11, 88} = 20.4$, $p < 0.01$), and an interaction effect was found between time and condition ($F_{22, 176} = 114.6$, $p < 0.01$). No significant differences were found between the three conditions when analysing each time point using a one-way ANOVA ($p \geq 0.10$); see Figure 3.

Satiation: There was no main effect of condition ($F_{2,14} = 1.83$, $p = 0.36$). There were main effects of time ($F_{11, 77} = 17.2$, $p < 0.01$). No interaction effect was found between condition and time ($F_{22, 154} = 1.57$, $p = 0.06$). Post - hoc analysis comparing time points in each condition back to baseline values revealed no main effects in either 10˚C ($p \geq 0.42$), 20˚C ($p \geq 0.11$) or 30˚C ($p \geq 0.15$); see Figure 4.

Fullness: A two-way repeated measures ANOVA revealed a main effect of condition ($F_{2,14} = 3.951$, $p = 0.04$), although post-hoc analysis using Bonferroni found no significant differences

between the three conditions ($p \geq 0.11$). A main effect was found for time ($F_{11, 77} = 21.53$, $p < 0.01$). Post-hoc analysis revealed significant differences compared to baseline in 20˚C ($p \leq 0.02$); see Figure 5. No significant differences compared to baseline were found in either 10˚C ($p \geq 0.06$) or 30˚C ($p \geq 0.15$). There was no condition by time interaction ($F_{22,154} = 1.14$, $p = 0.11$).

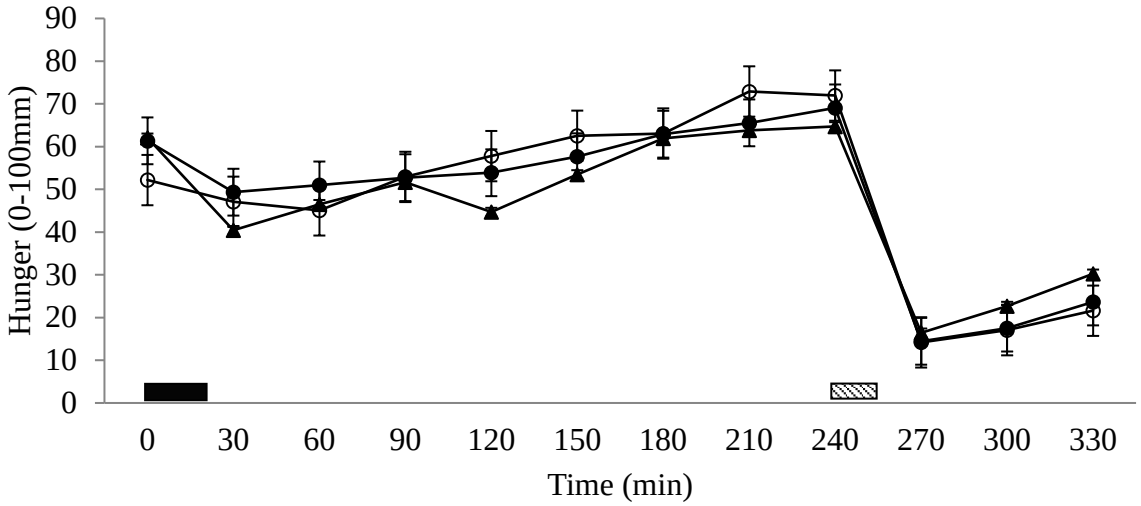

Figure 3 Hunger (mm) throughout each condition. Values presented as mean ± SE. ▲10˚C, ○ 20˚C, ● 30˚C, ▬ standardised meal, ▨ *ad libitum* meal.

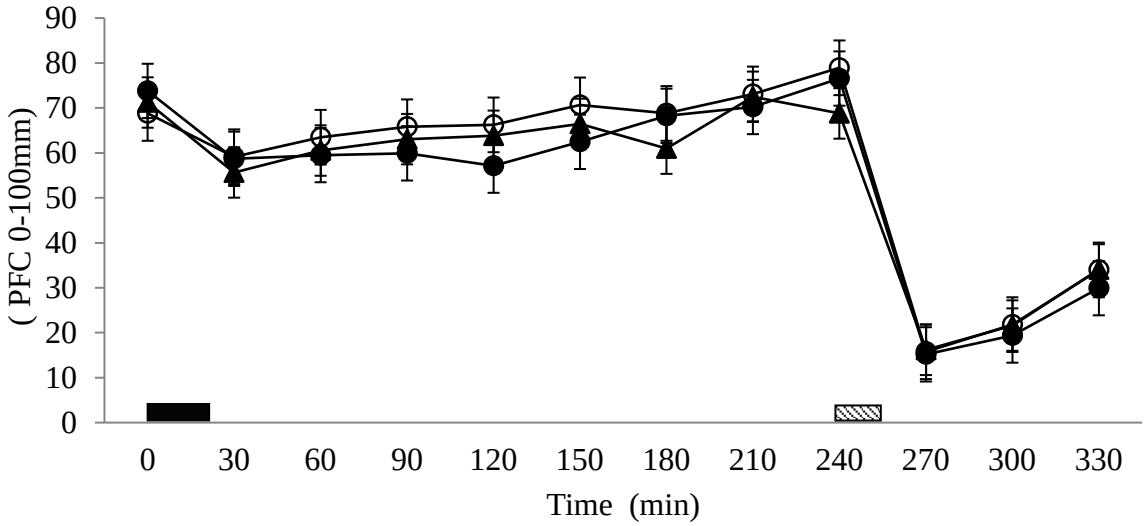

Figure 4 Mean PFC (mm) across each condition. Values presented as mean ± SE. ▲10˚C, ○ 20˚C, ● 30˚C, ▬ standardised meal, ▨ *ad libitum* meal.

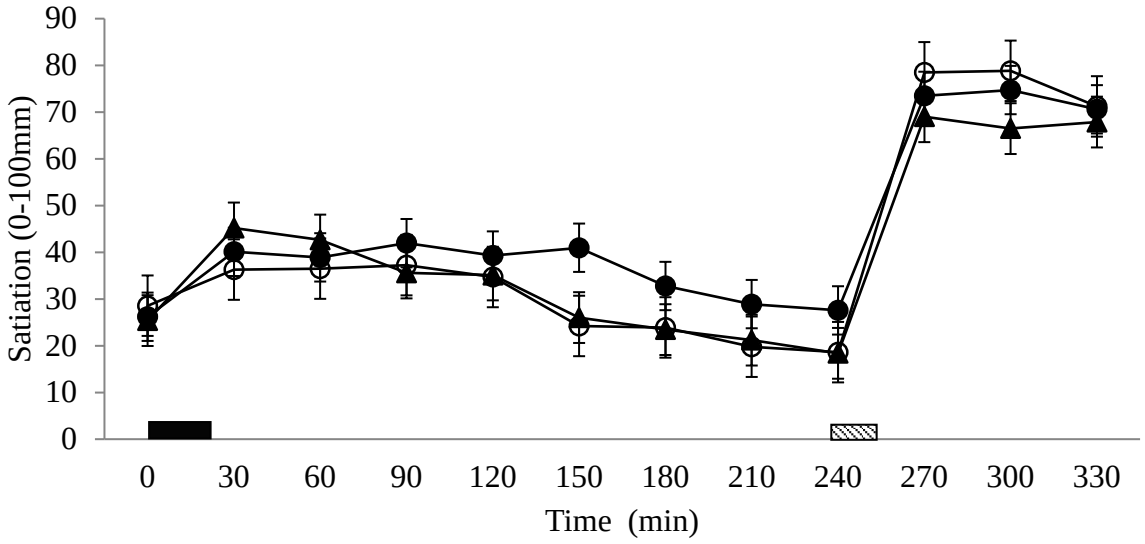

Figure 5 Satiation across all three conditions. Values presented as mean ± SE. ▲10˚C, ○ 20˚C, ● 30˚C, ▬ standardised meal, ▨ *ad libitum* meal.

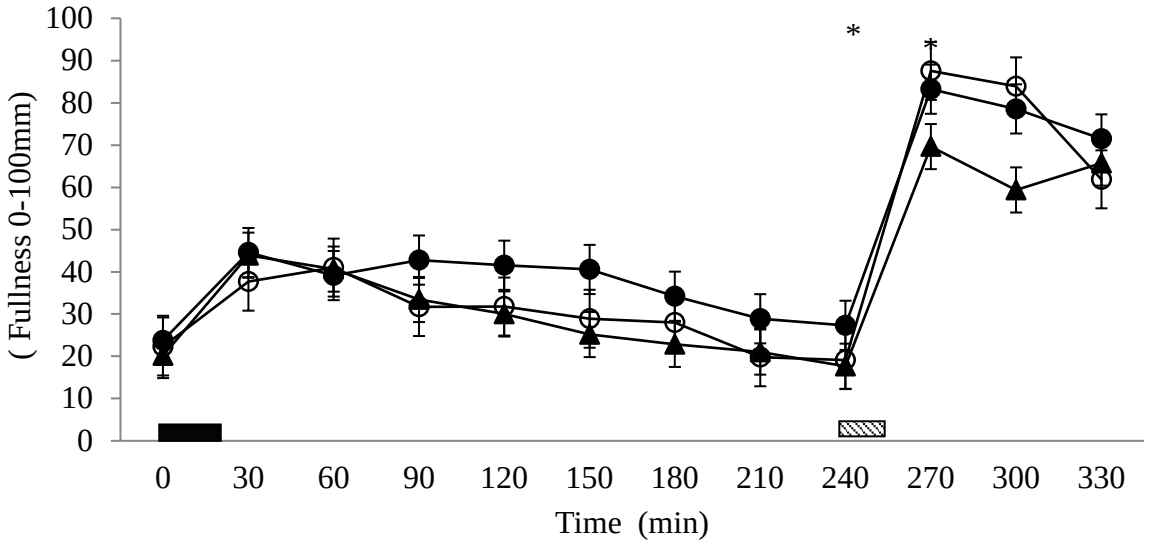

Figure 5 Fullness (mm) across each condition. * Significantly higher compared to baseline in 20˚C. Values presented as mean ± SE. ▲10˚C, ○ 20˚C, ● 30˚C, ▬ standardised meal, ▨ *ad libitum* meal.

Overall appetite: There was no main effect of condition for overall appetite *(p* = 0.42). There was a main effect of time for overall appetite (*p* < 0.01). Bonferroni post-hoc demonstrated significant differences compared to baseline, as shown in Figure 7. No significant condition by time interaction was found (*p* = 0.17).

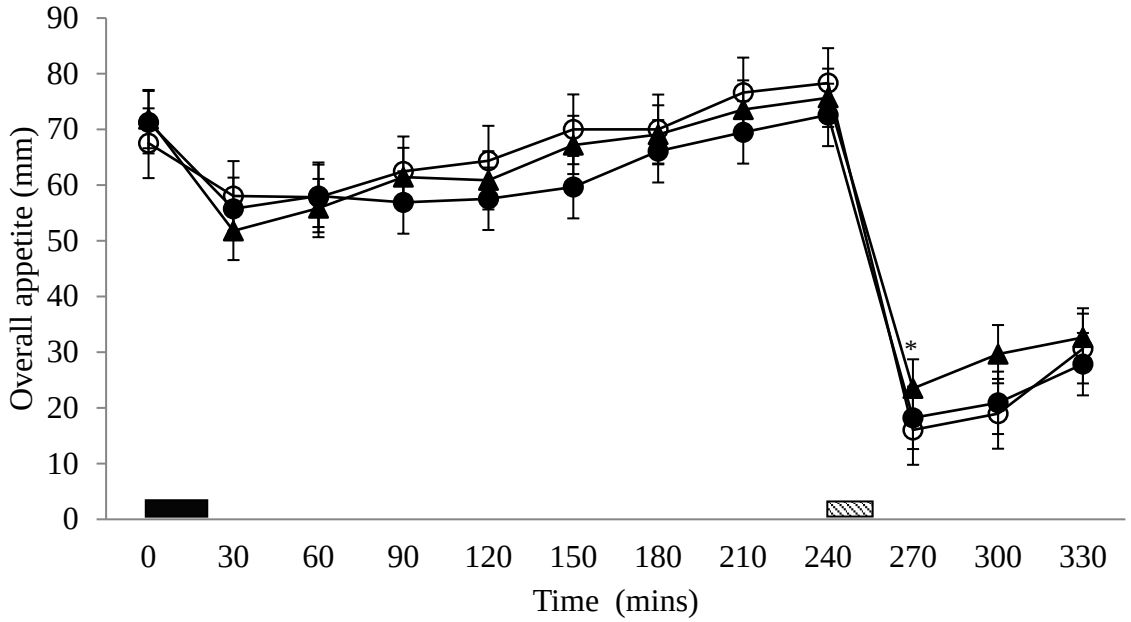

Figure 7 Overall appetite across conditions. Values are a calculation of all appetite variables ± SE (*n* = 9). * denotes significant decrease compared with baseline, in both 10˚C (*p* = 0.04), 20˚C (*p* = 0.03) and 30˚C (*p* = 0.03). ▲10˚C, ○ 20˚C, ● 30˚C, ▬ standardised meal, ▨ *ad libitum* meal.

4.2 *Ad libitum* energy intake

A one-way ANOVA revealed a main effect of condition for *ad libitum* energy intake ($F_{2, 16}$ = 9.92, p = 0.00). Post-hoc tests using Bonferroni correction demonstrated that energy intake was 266 kcal higher in 10˚C (p = 0.02) and 239 kcal higher in 20˚C (p = 0.01) when compared with 30˚C. There was no difference between 10˚C and 20˚C (p = 1.00) (see Figure 8).

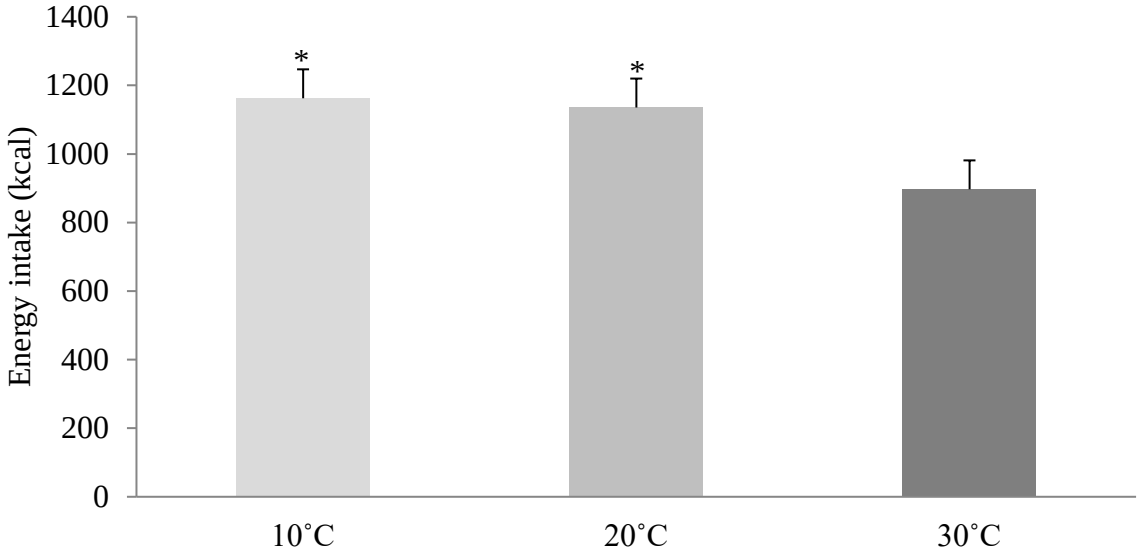

Figure 8 Energy intake (kcal) in the 10˚C, 20˚C and 30˚C conditions. * demonstrates significant increase from 30˚C.

4.3 Plasma acylated ghrelin concentrations

A two-way ANOVA revealed significant effects of condition for delta plasma acylated ghrelin concentrations (i.e., change from baseline) ($F_{2, 1.6}$ = 3.58), p = 0.05). Main effects were found for time ($F_{8, 64}$ = 5.62, p < 0.01). Additionally, a condition by time interaction was found ($F_{16, 128}$ = 3.80, p < 0.01. Follow up analysis revealed significant differences at 60 min between 10˚C and 20˚C (p < 0.0005) and 30˚C and 20˚C (p = 0.02). Significant effects were also found between 10˚C and 20˚C at time point 90 min (p = 0.01). At time point 330 min significant differences were found between 10˚C and 30˚C (p = 0.03). See Figure 9.

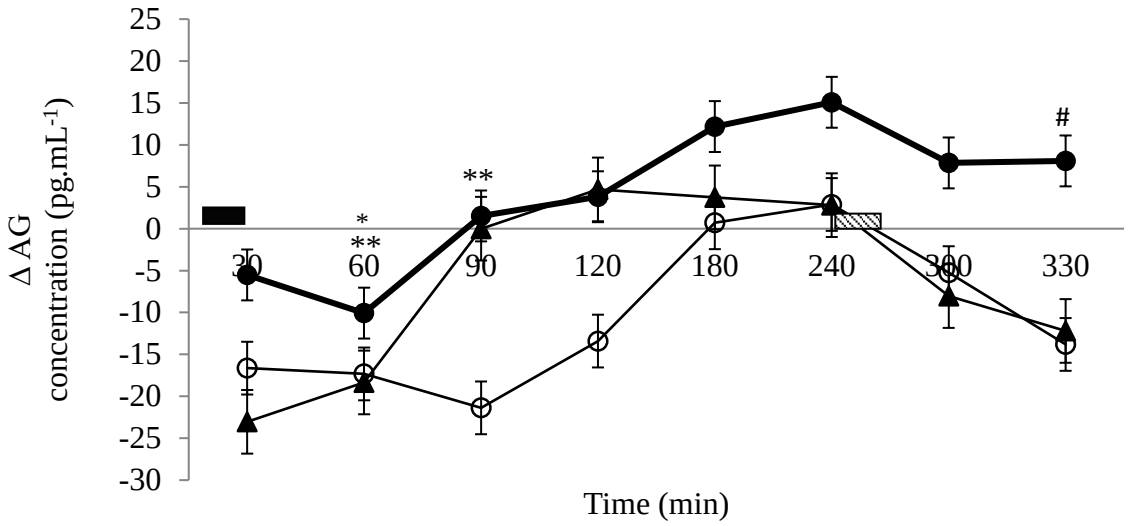

Figure 9 Delta plasma acylated ghrelin (AG) concentrations (pg.mL^{-1}) during each condition. Values are represented as mean ± SE (n = 9) * denotes significant difference between 30°C and 20°C. ** shows significant differences between 10°C and 20°C. # shows significant difference 10°C and 30°C. ▲10˚C, ○ 20˚C, ● 30˚C, ▬ standardised meal, ▨ *ad libitum* meal.

4.4 PYY concentrations

There was no significant main effect of condition for ΔPYY (i.e., change in PYY from baseline) ($F_{2, 16}$ = 1.85, p = 0.19). Main effects were found for time ($F_{8, 64}$ = 8.95, p < 0.05) and an interaction effect was found between time and condition ($F_{16, 128}$= 5.87, p < 0.05). Post-hoc analysis of each time point revealed significance between 30˚C (24.0 ± 91.6 pg.mL^{-1}) and 20˚C (-44.5 ± 64.8 pg.mL^{-1}) at only 300 min (p = 0.02) (see Figure 10).

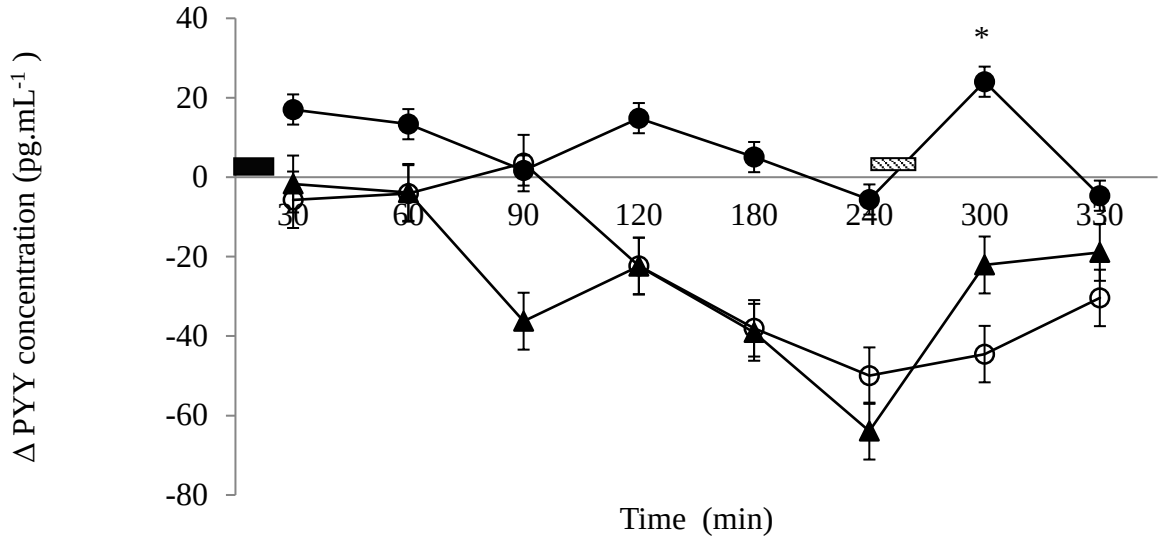

Figure 10 Plasma concentrations of ΔPYY (pg.mL^{-1}) for the three conditions across time. Values are Δ mean ± SE. * Denotes significant effects between 30˚C and 20˚C. ▲10˚C, ○ 20˚C, ● 30˚C, ▬ standardised meal, ▨ *ad libitum* meal.

4.5 Haematocrit

There were main effects of condition ($F_{2, 14}$ = 4.89, p = 0.02). Follow up with bonferroni found no significant differences between conditions ($p \geq 0.09$). No main effects of time ($F_{8, 56}$= 0.80, p = 0.60) and no condition by time interaction ($F_{16, 112}$= 0.91, p = 0.56) for haematocrit.

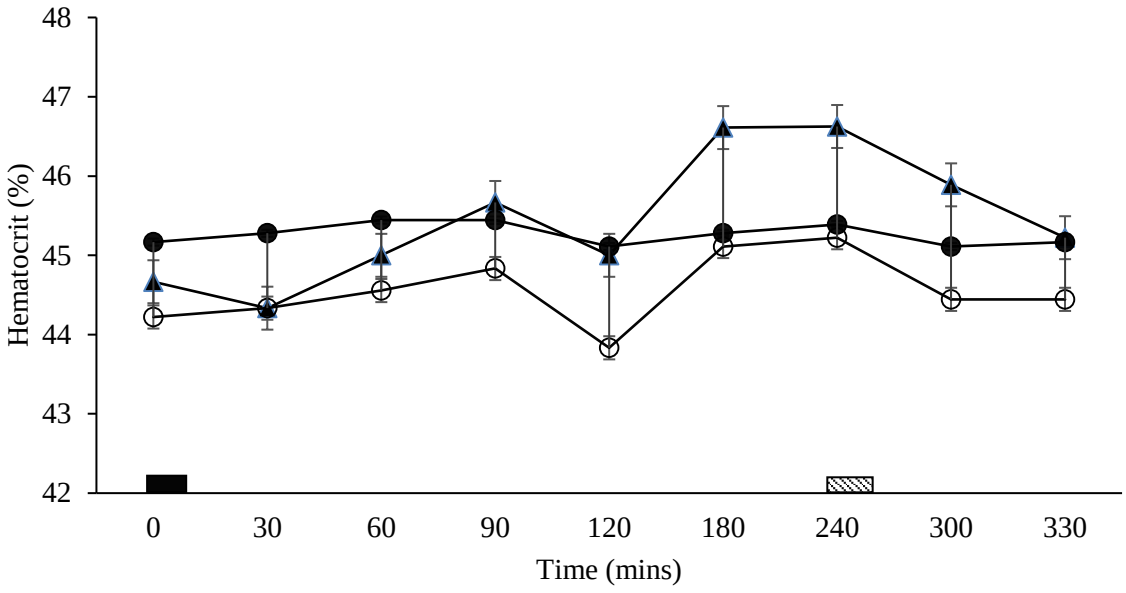

Figure 11 Hematocrit (%) for the three conditions across time. Values are Δ mean ± SE▲10˚C, ○ 20˚C, ● 30˚C. ▬ standardised meal, ▨ *ad libitum* meal.

4.6 Haemoglobin

There was no main effect of condition on haemoglobin ($F_{2, 14} = 0.94$, $p = 0.42$). Main effects were found for time ($F_{8, 56} = 0.68$, $p < 0.05$). Bonferroni follow up showed significant differences between 90 min and 180 min, with the latter being significantly lower ($p = 0.04$). An interaction effect was not found for time and condition ($F_{16, 112} = 1.55$, $p = 0.09$).

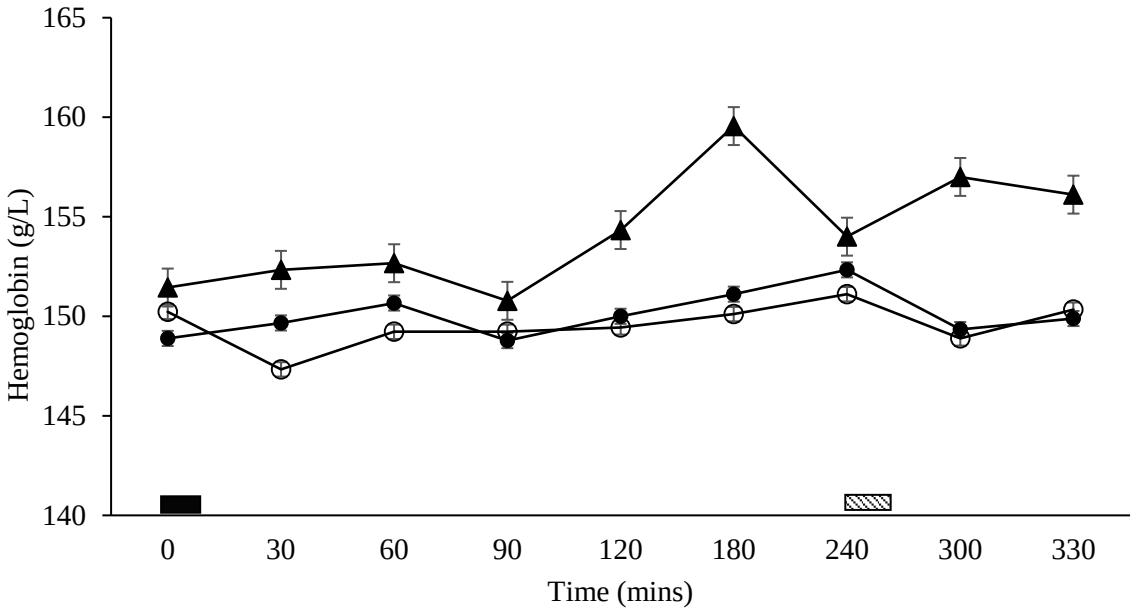

Figure 12 Haemoglobin (g/L) for the three conditions across time. Values are Δ mean ± SE▲10˚C, ○ 20˚C, ● 30˚C. ▬ standardised meal, ▨ *ad libitum* meal.

4.7 Physiological and thermoregulatory measures

4.7.1 Heart rate

The main effect of condition was not significant for heat rate ($F_{2, 14} = 7.19$, $p = 0.07$), but the main effect of time was and ($F_{14, 33} = 3.65$, $p < 0.01$), specifically between time 1.5h and 4h ($p = 0.04$). No interaction between condition and time was found ($F_{66, 462} = 48.95$, $p = 0.85$).

4.7.2 Core temperature

Statistical analysis revealed no main effects of condition for core temperature ($F_{2, 12} = 3.52$, $p = 0.63$). No main effects for time were found for core temperature ($F_{33,198} = 4.99$, $p = 0.35$). No interaction was found between condition and time ($F_{66, 396} = 0.53$, $p = 0.53$).

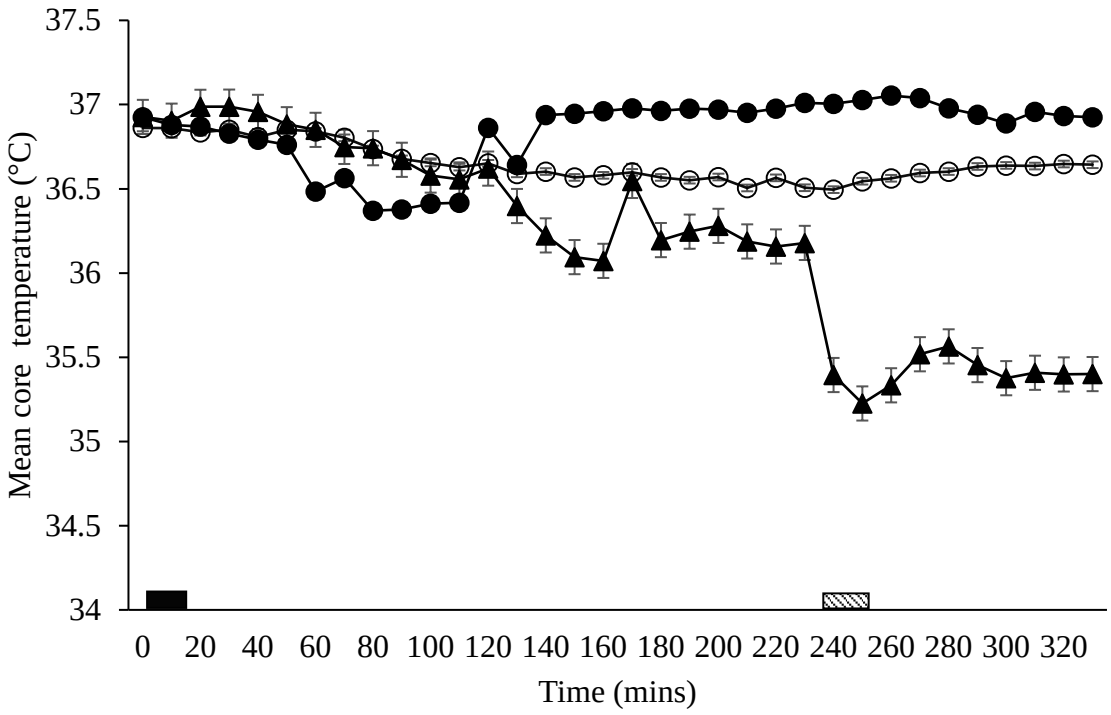

Figure 13 Core temperature (°C) for the three conditions. Values are Δ mean ± SE. ▲10˚C, ○ 20˚C, ● 30˚C. ▬ standardised meal, ▨ *ad libitum* meal.

4.7.3 Skin temperature

Statistical analysis revealed main effects for conditions for mean skin temperature ($F_{2,116}$ = 112.78, P <0.005). No effect of time ($F_{11,88}$ = 1.27, P = 0.26) was observed. There was a significant condition*time interaction ($F_{22,176}$ = 11.18, P < 0.005)

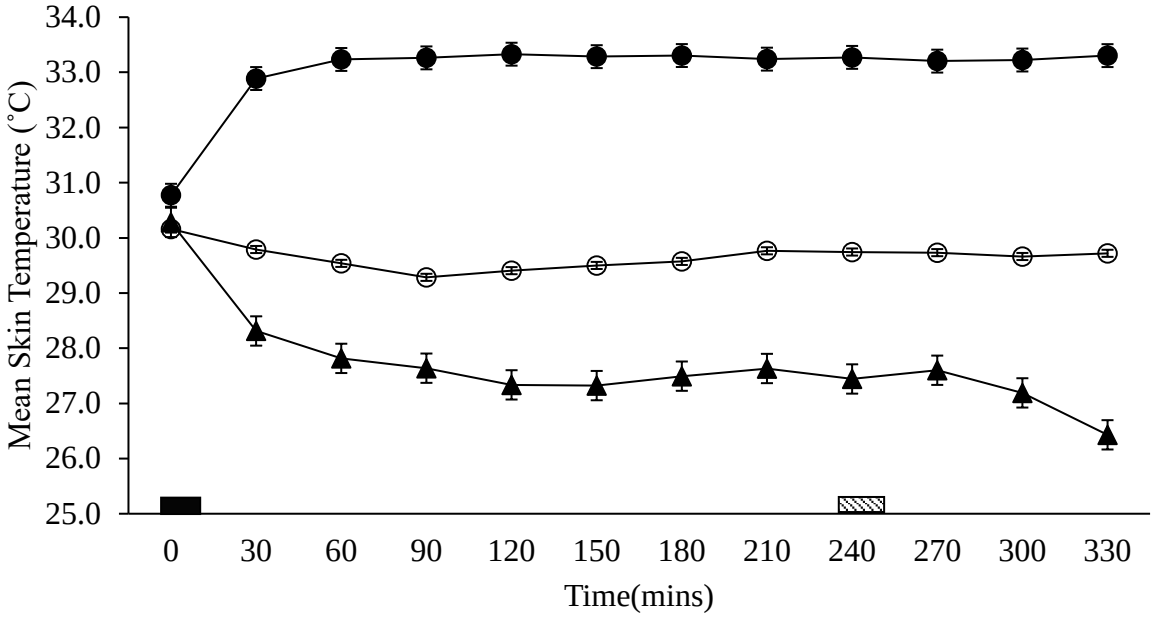

Figure 14 Skin temperature (°C) for the three conditions across time. Values are Δ mean ± SE. ▲10˚C, ○ 20˚C, ● 30˚C. ▬ standardised meal, ▨ *ad libitum* meal.

4.7.4 Body heat content

A two-way repeated measures ANOVA revealed there were main effects of condition for body heat content ($F_{2, 10}$ = 52.52, p < 0.05). Post-hoc analysis demonstrated significance was found between 10˚C (34.7 ± 0.1˚C) and 30˚C (35.9 ± 0.8˚C) (p = 0.01) and between 30˚C and 20˚C (35.1 ± 0.9˚C) (p < 0.01). Significant effects were not found across time ($F_{10, 33}$ = 1.09, p = 0.35). The condition by time interaction was not significant ($F_{66, 330}$ = 1.1, p = 0.27). See Figure 15.

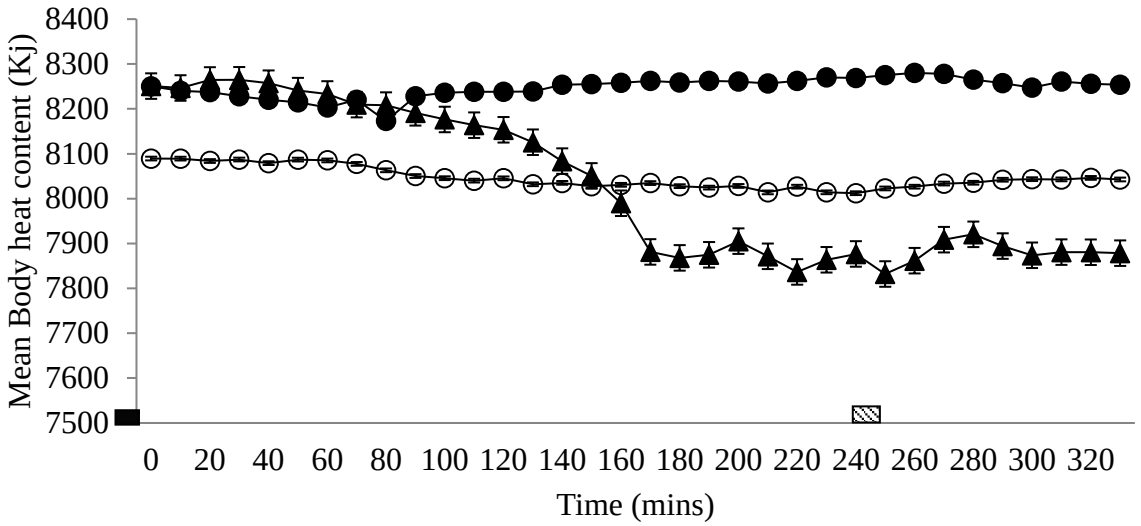

Figure 15 Body heat content for the three conditions across time. Values are Δ mean ± SE. ▲10˚C, ○ 20˚C, ● 30˚C. ▬ standardised meal, ▨ *ad libitum* meal.

4.7.5 Body weight changes

A one-way ANOVA revealed no main effects of condition for body weight changes over time for 10°C (0.65 ± 0.46 kg), 20°C (0.88 ± 0.59 kg) or 30°C (0.72 ± 0.64 kg) ($F_{2,16}$ = 0.60, p = 0.56).

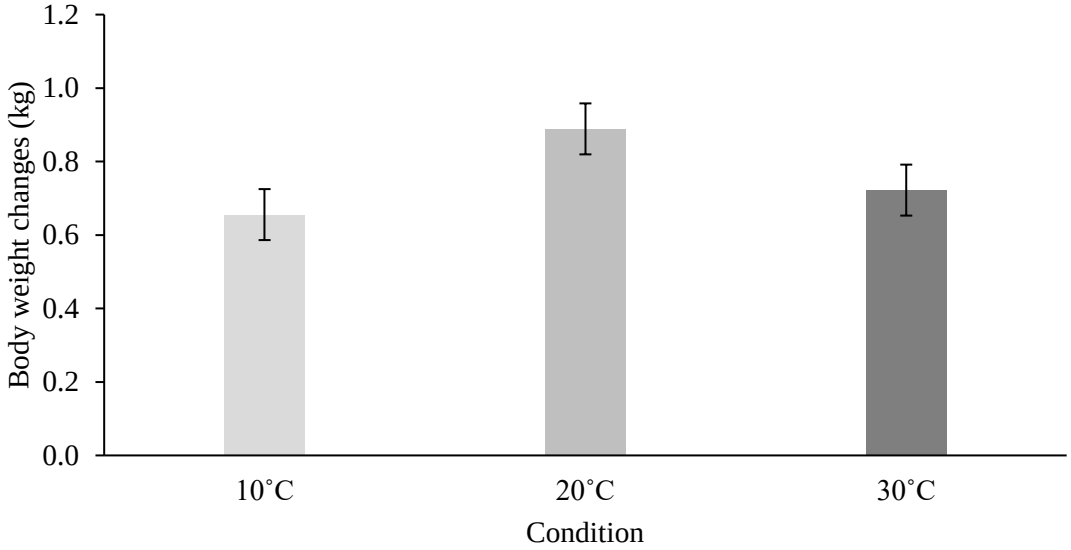

Figure 16 Body weight changes (Kg) for the three conditions. Values are Δ mean ± SE. ▲10˚C, ○ 20˚C, ● 30˚C.

4.7.6 Osmolality

A one-way ANOVA revealed no significant differences were found for condition 10˚C (370 ± 182.96 mOsm.kg H_2O), 20˚C (376.6 ± 128.55 mOsm.kg H_2O) or 30˚C (360 ± 178.54 mOsm.kg H_2O) ($F_{2, 24} = 0.14$, $p = 0.86$). See Figure 17.

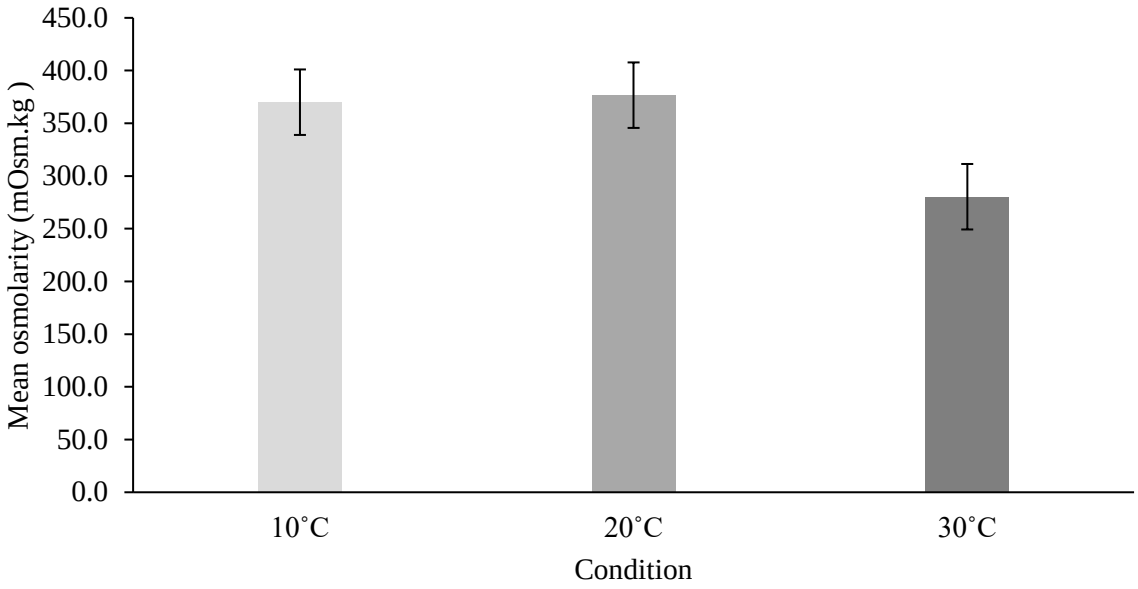

Figure 17 Osmolality (mOsm.kg) for the three conditions. Values are Δ mean ± SE▲10˚C, ○ 20˚C, ● 30˚C.

Chapter VI

Discussion

The purpose of the present study was to investigate the effects of environmental temperature on appetite, energy intake and concentrations of the appetite-regulating hormones: acylated ghrelin and PYY. Previous research within this field investigated the effects of different temperatures with the additional element of an exercise bout on appetite and appetite-regulating hormones. To the author's knowledge, the present thesis is the first which assesses the effects of an acute exposure to different environmental temperatures on appetite and appetite-regulating hormones during rest within a cold, thermoneutral and hot condition. The main findings are: 1) energy intake was significantly lower when resting in the hot environment compared to the cold and thermoneutral environments 2) no significant difference in overall appetite between the three conditions 3) there was a significant difference between delta acylated ghrelin in 30°C compared to 20°C and 10°C, finally 4) Delta PYY was higher in the hot condition at 300 min (5 h) compared with the thermoneutral condition.

Energy intake was lower in the hot condition compared to cold and thermoneutral conditions, with participants eating an average an addition of 239 kcal in the thermoneutral environment and 266 kcal in the cold environment when compared to the hot. Subsequently, the present study found that resting (for 4 h prior a meal) in a hot environment tends to decrease meal (lunch) energy intake compared to thermoneutral and cold conditions. This is in line with similar findings that revealed exposure to the cold increases energy intake (Westerterp-Plantenga *et al.*, 2002, Shorten *et al.*, 2009, Wasse *et al.*, 2013, Crabtree and Blannin, 2015). Nonetheless, the previous studies all included a bout of exercise and cannot be directly compared with the present study where the participants rested for the entire trial. Indeed, there is a large body of evidence showing that exercise decreases appetite (Martins *et al.*, 2008),

which may have influenced the effect of temperature on appetite in previous studies. Thus, the finding from the present study makes a novel contribution to the literature, suggesting that the increased energy intake observed after exercise in the cold in previous work may have occurred without exercise being performed. In this way, there appears to be an independent effect of environmental temperature on energy intake.

Preceding outcomes from Westerterp-Plantenga et al. (2002) support the results that hotter environments reduce energy intake. These authors' reported an increase in energy intake and subjective appetite whilst resting at 16°C compared to 22°C. However, this previous work used a narrower range of temperatures than the present study, which limits the application of the results to more extreme environmental temperatures. Nevertheless, the participants were exposed to each environment for 60 h; exposure to 10°C for this duration of time may not have been feasible due to feelings of discomfort and health and safety considerations. The authors concluded that overeating at a lower temperature compensates for an increase in energy expenditure as well as attenuating a decrease in core body temperature (Westerterp-Plantenga *et al.*, 2002). With these results in mind, it has been suggested that the resultant heat loss occurred in cold environments leads to compensatory strategies such as increasing food intake. This has been supported by studies reporting higher caloric needs in soldiers within Arctic settings where the average summer temperature is 3-12°C, whilst winter is -34°C (Swain *et al.*, 1949). This research concluded body weight increases were found by an average of 1.55 kg through a 20-week time period after daily cold exposure of 3 h, which was attributed to the thermic effect of food via energy intake endorsing the production of heat (Swain *et al.*, 1949). Conversely, when an environment is significantly hot, the need to dissipate heat increases and thus caloric intake reduces as this would promote heat production through the thermic effect of food. The temperature – dependent variation which has been found in this present study, in principle, should be reflected in appetite (Brobeck, 1948). However, it is not possible to directly

analogise the findings of the present study with those of (Swain *et al.*, 1949) which used exposure over a 20 week period in the Arctic, whereas the present study examined an acute 5.5 h duration of exposure within an environmental chamber. Additionally, the temperature was not controlled during the Arctic setting and may have varied considerably over the course of study. In contrast, environmental temperature was controlled and monitored continuously in the present study.

The reduction in energy intake at hot temperatures found in the present study is in discordance between previous findings whereby relative energy intake was reduced in a hot environment compared to a resting control (Shorten *et al.*, 2009). Nevertheless, it is in agreement with Wasse *et al.* (2013), where total energy intake was lower following a bout of exercise in a hot environment. Unlike the present study, Shorten *et al.* (2009) included a 40 min bout of exercise at 36˚C, 25˚C and a control of resting at 25˚C. Participants were only exposed to the each temperature for 40 min whereas the present study participants rested in the environmental temperature for 5.5 h. Furthermore, the previous study is limited as it did not include an assessment of subjective appetite, and energy intake was only measured once immediately post exercise. It may be logical that during succeeding meals, energy intake may have shown compensatory increases and therefore decreased the exercise – induced energy deficits that were found. Results from Wasse *et al.* (2013) illustrates participants on average consumed 1400 kJ less during buffet meals succeeding exercising in the heat (30°C) compared with temperate (20°C). However, the authors elaborate further that 60% of energy intake consumed was from the first buffet meal out of two (1 h post exercise and 4.5 h post exercise); this effect was similar between the two conditions. The authors specify that the tendency for a decreased energy intake during the hot trial compared with temperate, persisted for the afternoon meal. This was in spite of perceived appetite occurring analogously in both trials after the first meal until the cessation of the trial. Limitations of Wasse *et al.* (2013) investigation include the lack

of a control trial, consequently, no comparison could be made between exercising in the heat and a resting control condition. Despite these differences in study designs, both investigations suggest that heat exposure decreases energy intake. Conversely, King *et al.* (2013a) reported no difference in energy intake over a period of 6 h after exercise (60 min treadmill run, 72% of $\dot{V}O_{2max}$) compared to a resting control. Therefore, a compensatory increase may not have been shown in the long term – i.e., after around 7 days of exercise, compensatory increases in EI may have occurred. A future recommendation would be to examine the effects of long term exposure of hot environments on EI and weight loss.

Although the findings from the present study suggest that cold exposure in the long term may promote energy intake and thus increases in body mass, it should be noted that there are other adaptations to cold exposure that may oppose this effect. Recently, studies have found that a 10-day period of cold acclimation resulted in a highly significant brown adipose tissue (BAT) recruitment achieved by stimulating conversion of white cells to beige cells (Cypess and Kahn, 2010, van der Lans *et al.*, 2014). Brown adipose tissue is known to have the capacity to modulate energy balance by consuming large amounts of energy for thermogenesis (Yoneshiro *et al*, 2011; Saito *et al*, 2009; Van Marken Lichtenbelt *et al* 2009) by using glucose and fatty acids as fuel (Rothwell and Stock, 1982, Lowell and Spiegelman, 2000, Stanford *et al.*, 2013). A key role for brown adipose tissue in metabolic regulation, particularly during or following cold exposure, suggests that a lack of the tissue or reduction in its activity could have a role in obesity (Bartelt *et al.*, 2011,Stanford *et al.*, 2013, Trayhurn, 2016). A supporting study found cold-exposure enhances brown adipose tissue oxidative metabolism in six healthy adult humans; however, the use of frequent and chronic cold exposure is yet to be established (Quellet *et al*, 2012). With results from numerous studies BAT involved in cold- induced thermogenesis this alongside inverse relationships between BAT activity and adiposity; it may be implied that brown adipose tissue is a

major component of whole body energy expenditure and may be involved in the regulation of energy balance and body fat content in humans

The results from the present study reveal no significant differences in overall appetite between cold, hot or thermoneutral resting conditions. This is a novel finding; previous studies have been anecdotal in regards to comparing the effects of appetite in hot, cold and thermoneutral environments without the element of exercise (Daly, 2014). When comparing resting in all three conditions, none affected subjective ratings of hunger, prospective food consumption, satiation or fullness. These current findings are in discordance of those by Tomasik *et al.* (2005) who found increased feelings of hunger in a cold environment. Nonetheless, the two studies differed as there were many variations in study design, indeed the study by Tomasik *et al.* (2005) included a 30 minute bout of rest and environmental exposure – both land based. Furthermore, energy intake was not measured to provide a direct indication of whether the rise in hunger resulted in increases in energy intake. It is clear that the influence of environmental temperature on appetite during rest remains relatively unknown, with little research into the area.

A strength of the present study was the measurement of the appetite-regulating hormones acylated ghrelin and PYY to provide a mechanistic understanding of changes in energy intake between the conditions. Research relating to the appetite-regulating hormonal response to different environmental temperatures is limited to a small number of studies that have produced equivocal results. Furthermore, the majority of studies have focused on the effects of temperature alongside an exercise component. Disputing previous research (Shorten *et al.*, 2009, Wasse *et al.*, 2013, Crabtree and Blannin, 2015) the present study reported significant differences in acylated ghrelin concentrations when exposed to either a hot compared to cold and thermoneutral environments for up to 6 h post-exercise, alongside significant changes in energy intake measured via *ad libitum* occurring. This suggests that increases in food

consumption in colder environments may be associated with alterations in concentrations of acylated ghrelin. Although participants were exposed for a longer duration of 6 h Wasse *et al.* (2013), results cannot be directly compared to the present study due to the inclusion of a 60 min running exercise bout at the start of the trial. Furthermore separate groups of participants completed cold vs. thermoneutral and the hot vs. thermoneutral trials. Therefore, no comparison between hot and cold environments was made. Conversely, the present study found significant differences in acylated ghrelin between the three conditions. An unexpected finding was that acylated ghrelin levels were significantly higher in the hot environment after eating the *ad libitum* meal compared with the cold and thermoneutral conditions. Research shows that consuming food reduces concentrations of acylated ghrelin (Cummings *et al.*, 2001, Wren *et al.*, 2001,Williams and Cummings, 2005). A possible reason behind these surprising results is that less food was consumed in the hot environment. However, this is just a postulation and previous research on environmental temperature and acylated ghrelin has produced inconsistent results. With some research finding ghrelin concentrations increased in a cold environment compared to hot/ neutral (Tomasik *et al.*, 2005, Crabtree and Blanin, 2015) and others finding no difference in concentrations between hot, cold and neutral conditions (Kojima *et al*., 2015, Wasse *et al*., 2013). Therefore, further research that uses standardised test meals to directly examine the effect of environmental temperature on acylated ghrelin independent of differences in lunchtime energy intake is required.

Findings from the present study revealed minimal differences in total PYY concentrations between cold, hot and thermoneutal conditions. The findings indicate that total PYY concentrations are not affected within a hot condition during a 5.5h exposure despite decreased energy intake being found. This supports recent research from (Kojima *et al.*, 2015) who found no differences in PYY concentrations between hot, neutral and cool conditions. In contrast to the present study the temperatures were higher (36°C, 24°C, 12°C). As a result, this implies

that PYY concentrations do not differ throughout a range of temperatures. Additionally, the prior study only exposed participants for 30 minutes (when exercising); thus a longer duration within the environment may be needed to elicit effects on PYY concentrations.

Despite no condition effects on PYY concentrations, looking at Figure 9 directly, the hot condition has a higher change from baseline throughout the trial. In addition a condition time interaction was found: increased PYY concentrations in the hot trial compared to thermoneutral at 300 min (5h; 1h post *ad libitum* meal). As total PYY has been associated with signalling satiety (Le Roux and Bloom, 2005, Moran and Dailey, 2011) the current findings propose reductions in energy intake in the hot environment in the present study may have been mediated by changes in total PYY concentrations. In this respect, the present study supports other findings (Shorten *et al.*, 2009) where increases in total PYY concentrations were found in hot conditions compared to cold. Furthermore, the current study found decreases in concentrations of PYY in 10°C, although not significant, supporting previous studies where a decrease in PYY concentration following exercise in a cold condition compared to a thermoneutral condition was found (Wasse *et al.*, 2013,). Direct comparisons of the previous study and present cannot transpire due to different study designs. The prior research in this area (Wasse *et al.*, 2013,). , did not include resting trials in hot or cold conditions to compare directly to this current study.

Gastric emptying may have influenced appetite-regulating hormone concentrations as it has previously been associated with increases in acylated ghrelin concentrations after cold exposure (Stengel *et al.*, 2010) . However, this finding is based on rodents therefore cannot be directly compared with humans (Stengel *et al.*, 2010) . Previous research has suggested that the nutrients within the gut influence the production of peptide hormones (Cummings and Overduin, 2007). Hormones which have been found to be affected by gastric emptying regulation include acylated ghrelin and PYY. There has also been a suggestion that the rate of gastric emptying is dependent on meal size, with gastric emptying occurring at a faster rate

with a smaller meal (Delgado-Aros *et al.*, 2004). This may be a reason behind the concentrations of both acylated ghrelin and PYY in the current study as participants ate more in the cold condition, which may have impacted the rate of gastric emptying, therefore the appetite-regulating hormone concentrations. However, on average participants consumed only 50 g more food, which may not have been a substantial amount for a change gastric emptying to occur. It is clear that further research is required to ascertain the multifaceted effects of energy intake and appetite-regulating hormones in relation to gastric emptying.

It should be highlighted it is possible that environmental temperature could have affected energy intake in the present study through changes in hydration rather than appetite-regulating hormones. However, our data suggests that this was not the case. Indeed, haematocrit and haemoglobin were similar between conditions, supporting that the changes in concentrations of appetite-regulating hormones were not due to changes in plasma volume which may occur with dehydration, for example. Furthermore, osmolality did not differ throughout the three conditions, where participants were in a euhydrated state.

Core temperature was similar between the conditions in the present study despite large differences in environmental temperature. This finding was expected as core temperature is usually well maintained through homeostasis (Nakamura and Morrison, 2008). In contrast, body heat content, the product of mean body temperature and body heat capacity (Sawka *et al.*, 2007) and the use of this variable within appetite research is a novel measure. Body heat content was significantly lower in the cold environment compared to the hot and thermoneutral environments. This was alongside a reduction in skin temperature in the cold environment when compared to hot and thermoneutral. Thus, confirming that the environment was truly cold despite participants being permitted to wear different clothing throughout the trials. However, it should be noted that body heat content was not different between 30˚C (35.9 ± 0.8) and 20˚C (35.12 ± 0.9), potentially limiting the possibility for differences in appetite between

these conditions. Contrastingly, previous literature found significantly elevated core temperatures within a hot condition compared to temperate and cold (Shorten *et al.*, 2009, Kojima *et al.*, 2015). Nevertheless, the studies included exercise, which possibly explains the increase in core temperature found between conditions in comparison to the present study where none was found.

5.1.1 Limitations

There were limitations with the present study within this thesis, which should be addressed in future research. The homogenous nature of participants who were young, healthy males and recruited from within the sport and exercise science population at the University of Bedfordshire may hinder applying the findings to other populations; for example females, overweight/obese or older individuals. Therefore, further clarification is needed regarding appetite and appetite-regulating hormone differences between men and women.

Another potential limitation of the present study was the possible influence of clothing on the participants responses to different environmental temperatures, as participants were allowed to behaviourally thermo regulate via clothing. However, this approach was taken purposefully, as 10°C is not tolerable for many individuals over a prolonged period, and the reduction in skin temperature on the cranium/ face and hands was sufficient to create a cold environment. Thus, this approach is more representative of habitual exposures to different environmental temperatures, where clothing would be controlled. This is likely to occur in real life situations. Nevertheless, differences in body heat content were still shown between the trials, being significantly higher in the hot condition (8264±917.5 Kj) compared with thermoneutral (8031 ± 847.3Kj) and cold (7875 ± 730.08Kj) conditions. Thus, despite differences in clothing between the conditions, it appears that the environmental conditions were extreme enough to have an impact on body heat content.

A further limitation of the present study was that energy expenditure was not measured. As this is an important factor influencing energy balance, this would have been beneficial in supporting the results in the current study, particularly as cold exposure has been shown to increase energy expenditure previously (Westerterp-Plantenga *et al.*, 2002). Finally, the use of the pasta meal for the measurement of *ad libitum* energy intake may not be ideal due to factors such as food preferences and boredom associated with eating the same meal four times (Gregerson *et al*., 2008, Venti *et al*., 2010, Hogenkamp, 2012). However, the use of a counterbalances trial order minimised the possible influence of boredom when comparing between the conditions.

5.1.2 Future recommendations

Appetite is multifaceted – it is affected by numerous environmental, psychological, social, and cultural stimuli (Blundell, 2006). Other hormones which play a role in appetite regulation include insulin, glucose, GLP-1 and tonic regulators such as leptin. Future research may consider investigating the effects of different environmental temperatures on these hormones to gain a more detailed understanding of how they are involved in mediating differences in energy intake. Future recommendations include investigating the effects of different environments on obese/overweight individuals to assess whether similar findings will occur as there is a need for long term interventions to assess the possible impact of temperature on body mass and body composition. If so it may be beneficial to employ certain environmental temperatures for the use of weight loss/management.

Future studies should take into consideration examining the effects of environmental temperatures on differing population groups such as the comparison of females or overweight/obese individuals. Research is required to ascertain whether manipulations in environmental temperature can be used as a strategy to promote weight loss. However, the present findings show implications for obesity prevention, which is more effective than a cure.

Additionally, research has shown that obese individuals appear to have a blunted response compared with those that are non- obese. It is already coming to light that there may be between-sex differences in appetite responses, with research showing men significantly increase their energy intake compared to women (Hagobian *et al.*, 2013). However, most research shows women tend to increase their EI in response to exercise compared to men. In addition, men more consistently lose weight when on a long term exercise programme compared to women. This research indicates that women may possess more effective mechanisms to maintain body fat (Hagobian and Braun, 2010).

Additional recommendations include the monitoring of the participants mood. It is likely that throughout the course of a 5.5 hour trial that boredom will occur from time to time, this can in turn affect appetite and therefore energy intake. The addition of temperature and its effect on mood and food consumption is unknown therefore the mediating relationship between temperature and energy intake needs to be established.

Ancillary future recommendations include the length of exposure. The current study used an acute exposure – examining the influence of environmental temperature over the long term would be needed to establish the possible effects on body weight, or whether there is an acclimation response; where people acclimatised to the heat do not show reductions in energy intake.

The current study did not measure energy expenditure, which is a fundamental component of energy balance. It is possible that energy expenditure may have been higher in the cold environment - this is supported by previous studies. However, the increase in energy expenditure may not be high enough to counteract the 239 kcal or 266 kcal increase in energy intake found in the current study. This needs to be accounted for in future investigations, furthermore future research should consider using diet diaries and accelerometers to examine energy intake and energy expenditure in free-living conditions post-exposure to the condition.

5.1.3 Application of findings

The current findings suggest that hot environments attenuate energy intake. Importantly, these effects were seen even during a relatively acute exposure of 5.5 h, with an *ad libitum* lunch being provided 4 hours into each exposure. Thus, manipulations in environmental temperatures may be beneficial to restrict energy intake for overweight and obesity management, for example, increased room temperature of an office. The implications for preventing overweight/obesity will lead to reducing the associated health and medical costs involved.

5.1.4 Conclusion

In conclusion, this study demonstrates that resting in the heat decreases energy intake compared to thermoneutral and cold environments. However, no differences were found in perceived appetite. Further research is required to confirm the role of gut hormones in explaining the link between environmental temperature and appetite, with the present study providing an unexpected findings of increased acylated ghrelin concentrations in the heat or increased total PYY mediating reductions in energy intake in the heat as concentrations were similar between the three conditions. Furthermore, the sample size should be addressed; having more than nine participants would increase the statistical power and may result in further significant effects. In addition, this line of research requires examination in diverse populations (e.g., overweight and obese). Ultimately, these findings could have implications for obesity prevention, as EI is a fundamental component for overweight and obesity management.

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Appendices

Appendix A

INFORMATION SHEET - STUDY 1

The effect of environmental temperature on appetite and appetite-regulating hormones during rest.

Dear Participant,

Thank you for showing an interest in participating in the study. Please read this information sheet carefully before deciding whether to participate. If you decide to volunteer, we thank you for your participation. If you decide not to take part, there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the aim of the project?

The purpose of the study is to investigate whether different environmental temperatures affect appetite and appetite-regulating hormones at rest. This study is being undertaken as part of the requirements of a Sport and Exercise Science MSc by research.

What type of participant is needed?

Males aged between 18-30 years who are not heat acclimated. A health screen questionnaire will be completed by each individual who volunteers to participate in the study. Those with the following conditions will be excluded from the study for their own safety: musculoskeletal injury that has affected normal movement within the last month, disturbance of vision, congenital heart disease, uncontrolled exercise-induced asthma, diabetes, epilepsy and chronic obstructive pulmonary disease (COPD). As it is not feasible to list every medical condition, it is possible that those with other medical conditions, not given above, may be excluded from the study once identified.

What will participants be asked to do?

Participants will be required to attend the laboratory on 4 separate occasions. The first visit for the preliminary tests will include a measurement of body composition using the Bodpod. For sessions 2-4, you will be asked to attend the laboratories in a fasted state (no food consumed 12 h prior to visit) and rest within different environmental conditions - thermoneutral (20°C), cold (10°C) and hot (30°C) conditions for a maximum period of 6 hours. Throughout visits 2-4, heart rate, thermal sensation, blood and subjective feelings

of hunger and temperature will be measured and recorded for each participant. For the measurement of the participants core temperature a rectal thermometer will need to be used to obtain this measurement. You will also be provided with a standardised breakfast and an *ad Libitum* meal during visits 2-4. Please also note that you will be required to record your food and drink intake the day before visit 2 and replicate this the day before visits 3 and 4.

What are the possible risks of taking part in the study?

Due to the nature of the study, participants will not be place under any unnecessary physical or mental stress throughout the duration of the study.

- Participants – Participants will be informed of the study and what they can do. A consent form will be completed before test measurements commence. Data collected will be either locked in a filing cabinet by a University member of staff or either in a password protected folder on a computer.

- Anonymity – The data collected would not in any way be linked to specific participants.

- During visit 1. Claustrophobia may occur when inside the Bodpod. However the participant will be made aware of the emergency release button.

- Blood sampling - A certified first aider will be on-site whilst blood sampling occurs and all procedures will be given special care. Samples will be collected in a clean and sterile environment to avoid the chance of infection and all wounds will be treated until bleeding has stopped and then covered to reduce the risk of infection.

- Exposure to extreme heat or cold – Core temperature will be monitored throughout all trials using a rectal thermometer to prevent this reaching dangerous levels. Please note that there are risks associated with of inserting anything into the body can cause shock (e.g., the rectal probe); specific safety precautions are carried out in case of any medical emergencies to ensure the participant is safe

What if you decide you want to withdraw from the project?

If, at any stage you wish to leave the project, then you can. There is no problem should you wish to stop taking part and it is entirely up to you. There will be no disadvantage to yourself should you wish to withdraw.

What will happen to the data and information collected?

Everyone that participates in the study will receive their own results for the tests that they complete. All information and results collected will be held securely at the University of Bedfordshire and will only be accessible to related University staff. Results of this project

may be published, but any data included will in no way be linked to any specific participant. Your anonymity will be preserved.

What if I have any questions?

Questions are always welcome and you should feel free to ask myself, Rachel Horsfall; Dr Julia Zakrzewski (Supervisor: Julia.Zakrzewski@beds.ac.uk) or Dr. John Hough (Second Supervisor: John.Hough@beds.ac.uk) any questions at anytime. See details below for my contact details.

Should you want to participate in this study then please complete the attached consent form, which needs to be returned before commencing the study.

This project has been reviewed and approved by the Ethics Committee of the Department of Sport and Exercise Sciences.

Many Thanks,

Rachel Horsfall

MSc by Research student

rachel.horsfall@study.beds.ac.uk

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Appendix B

CONSENT FORM - STUDY TWO

TO BE COMPLETED BY PARTICIPANT

NAME: …… (Participant)

I have read the Information Sheet concerning this project and understand what it is about. All my further questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:

 My participation in the project is entirely voluntary and I am free to withdraw from the project at any time without disadvantage or prejudice.

 I will be required to attend 4 sessions in the laboratory (1 familiarisation, 3 for main trials) to complete the project.

As part of the study I will have to:

 Insert a rectal thermometer to measure core temperature

Participants will not be able to leave the chamber, therefore if needed, I will be asked to urinate behind a screen within the environmental chamber.

 Give blood samples to be analysed for a number of hormones

 Have limited exposure to food cues, e.g. food based television programmes or films.

I am aware of any risks that may be involved with the project.

All information and data collected will be held securely at the University indefinitely. The results of the study may be published but my anonymity will be preserved.

Signed …… (Participant) Date: ……

Appendix C

University of Bedfordshire

Sport & Exercise Science Laboratories
Polhill Avenue
Bedford MK41 9EA

PRE-TEST MEDICAL QUESTIONNAIRE

To be completed by all subjects before participating in practical sessions.

Name: .....
Age:..... Gender: M / F

1 Are you in good health? Yes / No
If no, please explain:

2 Are you pregnant or have you given birth in the last 6 months? Yes / No

3 How would you describe your present level of moderate activity?
< once per month
once per month
2-3 times per week
4-5 times per week
> 5 times per week

4 Have you suffered from a serious illness or accident? Yes / No
If yes, please give particulars:

5 Are you recovering from an illness or operation? Yes / No
If yes, please give particulars:

6 Do you suffer, or have you ever suffered from:
Respiratory conditions (asthma, bronchitis, tuberculosis, other)? Yes / No
Diabetes? Yes / No
Epilepsy? Yes / No
High blood pressure? Yes / No
Heart conditions or circulation problems:
(angina, high blood pressure, varicose vein, aneurysm, embolism, heart attack, other)?
Do you have chest pains at any time? Yes / No
Do you suffer from fainting/blackouts/dizziness? Yes / No
Is there any history of heart disease in your family? Yes / No

7 Are you currently taking medication ? Yes / No
If yes, please give particulars:

8 Are you currently attending your GP for any condition or have you consulted your doctor in the last three months? If yes, please give particulars: Yes / No

9 Have you had to consult your doctor, or had hospital treatment within the last six months? Yes / No

10 Have you, or are you presently taking part in any other laboratory experiment? Yes / No

11. Are you currently fitted with a pacemaker? Yes / No

12. Do you have any food allergies or intolerances? Yes / No

If yes, please state what this allergy or intolerance is.....

PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to do the experimental exercise task if they:

- have a fever, suffer from fainting spells or dizziness;
- have suspended training due to a joint or muscle injury;
- have a known history of medical disorders, i.e. high blood pressure, heart or lung disease;
- have had hyper/hypothermia, heat exhaustion, or any other heat or cold disorder;
- have anaphylactic shock symptoms to needles, probes or other medical-type equipment.
- have chronic or acute symptoms of gastrointestinal bacterial infections (e.g. Dysentery, Salmonella)
- have a history of infectious diseases (e.g. HIV, Hepatitis B); and, if appropriate to the study design, have a known history of rectal bleeding, anal fissures, haemorrhoids, or any other condition of the rectum;

DECLARATION

I hereby volunteer to be a subject in experiments/investigations during the period of 20___.

My replies to the above questions are correct to the best of my belief and I understand that they will be treated with the strictest confidence. The experimenter has explained to my satisfaction the purpose of the experiment and possible risks involved.

I understand that I may withdraw from the experiment at any time and that I am under no obligation to give reasons for withdrawal or to attend again for experimentation.

Furthermore, if I am a student, I am aware that taking part or not taking part in this experiment, will neither be detrimental to, or further my position as a student.

I undertake to obey the laboratory/study regulations and the instructions of the experimenter regarding safety, subject only to my right to withdraw declared above.

Name of subject (please print) _____

Signature of Subject _____ Date: _____

Name of Experimenter (please print) _____

Signature of Experimenter _____ Date: _____

Appendix D

BLOOD ANALYSIS – Participant Screening Form

Please read the following:

a. Are you suffering from any known active, serious infection?

b. Have you had jaundice within the previous year?

c. Have you ever had any form of hepatitis?

d. Have you any reason to think you are HIV positive?

e. Have you ever been involved in intravenous drug use?

f. Are you a haemophiliac?

g. Is there any other reason you are aware of why taking blood might be hazardous to your health?

h. Is there any other reason you are aware of why taking your blood might be hazardous to the health of the technician?

Can you answer **Yes** to any of questions a-g? Please tick your response.

Yes ☐ No ☐

Small samples of your blood (from finger or earlobe) will be taken in the manner outlined to you by the qualified laboratory technician. All relevant safety procedures will be strictly adhered to during all testing procedures (as specified in the Risk Assessment document available for inspection in the laboratory).

I declare that this information is correct, and is for the sole purpose of giving the tester guidance as to my suitability for the test.

Signed …….

Date …….

If there is any change in the circumstances outlined above, it is your responsibility to tell the person administering the test immediately.

Appendix E

Breakfast Habits Questionnaire

Please read every question carefully. Choose the box that fits your answer best and fill it in. This is not a test so there are no wrong answers. Also, nobody who knows you will look at your questionnaire once you have finished it.

1. **How often do you usually have breakfast?** Mark one box for weekdays and one box for weekend.

Weekdays (Mon-Fri)

- ☐ I never have breakfast on weekdays
- ☐ One day
- ☐ Two days
- ☐ Three days
- ☐ Four days
- ☐ Five days

Weekend (Sat-Sun)

- ☐ I never have breakfast on the weekend
- ☐ I usually have breakfast on only one day the weekend (Saturday OR Sunday)
- ☐ I usually have breakfast on both weekend days (Saturday AND Sunday)

2. **What time do you normally have breakfast?**

Weekdays ☐☐:☐☐ AM / PM

Weekend ☐☐:☐☐ AM / PM

3. **Where do you normally have breakfast?** Mark one box for weekdays and one box for weekend.

Weekdays

- ☐ At home
- ☐ On way to work/school/University
- ☐ Other: _____

Weekend

- ☐ At home
- ☐ On way to work/ school/University
- ☐ Other: _____

4. **What do you normally eat and drink for breakfast?** Please provide details next to the relevant category.

Weekdays

☐ Ready-to-eat cereal

- Whole grain_____
- Refined grain_____

☐ Cooked cereal (e.g. porridge) _____

☐ Bread/Toast

- Type of bread: _____ Spreads _____
- Other details _____

☐ Meat/fish/eggs _____

☐ Other _____

☐ Drinks

- Hot drinks (e.g. tea, coffee)_____
- Cold drinks (e.g. pure fruit juice, milk) _____

Weekends

☐ Ready-to-eat cereal

- Whole grain _____
- Refined grain_____

☐ Cooked cereal (e.g. porridge) _____

☐ Bread/Toast

- Type of bread: _____ Spreads _____
- Other details _____

☐ Meat/fish/eggs _____

☐ Other _____

☐ Drinks

- Hot drinks (e.g. tea, coffee)_____
- Cold drinks (e.g. pure fruit juice, milk) _____

5. If you ever skip breakfast, why do you skip breakfast? Mark one box for weekdays and one box for weekend.

Weekdays	**Weekend**
☐ Not hungry (lack of appetite)	☐ Not hungry (lack of appetite)
☐ Feel nauseated/ weak/ tired	☐ Feel nauseated/ weak/ tired
☐ Do not like the food	☐ Do not like the food
☐ No motivation to prepare breakfast	☐ No motivation to prepare breakfast
☐ Lack of time	☐ Lack of time
☐ Would rather sleep	☐ Would rather sleep
☐ To help lose weight	☐ To help lose weight
☐ Other: _____	☐ Other: _____

6. Do you smoke?

☐ Yes ☐ No

7. Have you smoked previously? If 'yes', please insert approximate dates.

☐ Yes, from _____ to _____ ☐ No

Appendix F

FOOD DIARY INSTRUCTIONS

- **Everything** that you eat and drink over the course of the testing should be **recorded** in this **diary** and **photographed**.

- In the evening, you can **look through the photos** you took that day and use them to help you complete your food diary.

- If you **forgot to take a photo** of something you ate or drank in the day, you should still **add this** to the diary.

- Please make sure you fill in all the columns for each food/drink item:

    1. **Date and time of day** – the date and time you had the food/drink (you only need to write the date at the beginning of each day).
    2. **Description – as much detail as possible. Please tell us the manufacturer's name (e.g. Kelloggs, Heniz) and cooking method (e.g. grilled, roast, boiled).**
    3. **Amount** – approximate portion or weight, most snack foods will have the weight of the food on the packet so you can write this in your diary (e.g. full packet of crisps).
    4. **Leftovers** – the amount that you did not eat or drink (e.g. apple cores, crusts of bread). **Make sure that all left over food is also photographed.**

- This information is important for understanding our results from the study, so it is very important that you **avoid missing things out or making it up!** Thank you!

Date and time of day	Brand name (e.g. Heinz, Tesco, Kel-logs)	Detailed description of food/drink and cooking method (e.g. boiled potatoes, canned sweetcorn, bacon fried in sunflower oil)	Amount served (grams)	Leftovers (grams)

Appendix G

Subject Number:_____ Trial_____ Date:_____

Visual Analogue Scale

Time Point: **Time:** **Temp:** **Humidty:**

Please indicate how hungry you are now by circling a relevant number

Not Hungry Fairly Hungry Hungry Very Hungry

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Place a mark on the horizontal lines below after considering the following questions:

How hungry do you feel?

I am not hungry at all ——— I have never been more hungry

How satisfied do you feel?

I am completely empty ——— I cannot eat another bite

How full do you feel?

Not at all full ——— Totally full

How much do you think you can eat?

Nothing at all ——— A lot

Appendix H

Descriptive statistics

Table Descriptive data for each variable across three conditions.

	Time (min)						
Variable	**0**	**60**	**120**	**180**	**240**	**300**	**330**
Hunger (mm)							
10°C	66.4±28.5	47.8±23.3	46.5±280	61.1±27.4	64.8±29.1	18.7±16.9	27.6±24.7
20°C	51.4±26.2	44.3±21.8	55.2±25.5	61.8±21.7	71.0±15.5	14.1±7.8	18.3±7.5
30°C	66.5±18.5	53.3±32.0	53.0±30.7	62.0±23.7	69.8±23.7	16.5±15.5	22. 5±20.1
PFC (mm)							
10°C	71.1±17.62	60.4±18.5	63.8±19.9	60.7±24.1	68.9±28.7	21.7±16.7	34.1±24.7
20°C	68.8±21.8	63.7±21.1	66.3±20.0	68.7±18.8	79±8.8	21.7±13.7	34.0±19.2
30°C	73.8±10.5	59.4±27.8	57.2±27.0	68.3±20.4	76.4±14.9	19.3±15.3	30.1±24.1
Satiation (mm)							
10°C	26.0±25.8	43.6±20.8	36.3±19.4	23.5±14.0	18.4±10.8	69.3±28.0	70.1±24.0
20°C	28.0±26.5	36.4±22.6	35.6±23.2	23.5±12.3	19.0±9.9	81.4±10.1	75.1±11.7
30°C	27.7±22.5	40.0±28.1	39.9±25.7	34.1±22.3	27.4±19.3	76.4±19.4	72.1±23.1
Fullness (mm)							
10°C	17.8±11.5	39.6±15.0	29.6±16.2	23.1±12.2	17.8±9.3	61.3±32.6	66.9±20.8
20°C	21.0±20.3	38±21.2	33.0±20.2	27.5±18.1	19.5 ±15.7	86.1±10.6	64.4±25.4
30°C	25.5±24.1	40.25±29.1	42.4±26.4	35.75±23.04	27±20.3	81±19.9	74.25±24.8
∆Acylated ghrelin(pg.mL^{-1})							
10°C		-18.3±14.8	19.5±14.6	5.4±19.4	0.4±13.6	-11.6±29.4	-15.7±22.7
20°C		-17.3±12.4	-13.4±20.4	0.7±19.0	2.9±19.1	-5.2±38.6	-13.8±12.1
30°C		-10.1±17.5	3.8±22.7	12.2±15.8	15.1±24	7.8±20.2	8.1±23.1
∆PYY(pg.mL^{-1})							
10°C		-3.8±112.1	-22.4±63.2	-39.0±101.7	-63.9±113.2	-22.1±102.4	-18.9±114.9

20°C		-4.1±39.0	-22.3±60.6	-38.0±50.5	-49.±54.9	-44.5±64.9	-30.4±97.9
30°C		13.3±51.5	14.9±33.8	5.1±28.2	-5.6±55.4	24.0±91.6	-4.7±48.02
Hematocrit (%)							
10°C	44.5±1.4	45.0±1.7	45±1.51	46.2±2.3	46.62±1.8	45.5 ±1.5	45.1±1.6
20°C	44.1±2.5	44.6±2.5	43.6±1.7	44.4±2.5	44.75±1.4	44.1±2.0	44.4±2.0
30°C	44.9±1.9	45.1±2.4	45.1±2.3	44.7±2.6	44.81±2.6	44.6±2.7	44.6±3.1
Hemaglobin(g/L)							
10°C	150.9±8.4	151.6±11.8	152.2±12.5	156.9±14.2	154±12.5	155.3±11.0	154.3±10.6
20°C	149.7±11.4	148.7±10.3	148.9±9.9	149.9±10.9	151.2±10.4	148.6±9	150.4±10.4
30°C	148.0±8.3	150.1±7.9	149.1±8.7	149.9±9.1	150.4±8.8	147.1±7.2	148.5±9.5
HR (Bpm)							
10°C	67.2±7.4	69.1±10.1	70.8±10.2	60±8.6	69.3±7.1	68.4±6.8	70.2±9.1
20°C	66.9±10.6	67.7±9.4	66±5.7	62±8.6	61.2±5.2	65.8±5	67.3±7.3
30°C	66.3±7	73±16.4	75.8±7.1	65.4±8.4	73.6±14	75.6±11.4	78.1±10.9
Tc (°C)							
10°C	36.9±0.3	36.8±0.2	36.6±0.3	36.3±0.4	36.2±0.5	36.1±0.5	36.2±0.6
20°C	36.9±0.2	36.8±0.2	36.6±0.2	36.4±0.4	35.05±4.1	36.6±0.4	36.6±0.4
30°C	36.9±0.2	36.7±0.2	36.8±0.2	37.0±0.2	36.95±0.2	36.8±0.7	36.0±2.2
Tsk (°C)							
10°C	30.3±1.6	27.81±1.2	27.33±1.1	27.5±1.2	27.4±1.2	27.2±2.3	26.4±2.4
20°C	30.2±0.9	29.5±0.7	29.4±0.8	29.6±0.8	29.7±0.8	29.7±1.0	29.8±1.0
30°C	30.8±1.1	33.2±0.6	33.3±0.5	33.3±0.5	33.3±0.6	33.22±0.5	33.3 ±0.6
BHC (Kj)							
10°C	8089.3±818.9	8233±894.3	80005±774.4	7868±737.1	7877±733.6	7874±734.5	7878±734.8
20°C	8250.8±899.11	8085±821.7	8045±839.3	8014±843.6	8012±848.9	8043±848.5	8045±849.9
30°C	8250.3±892.6	8146±832.22	8238±907.2	8259±893.63	8269±900.96	8247±950.61	8254±929.95

Descriptive data are mean values ± standard deviation during the 5.5h exposure period.